

**The Effects of Branched Chain Amino
Acid Supplementation on Indicators
of Muscle Damage after Prolonged
Strenuous Exercise**

by

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requirements for the degree of
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DECLARATION

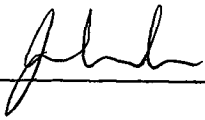
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EDUCATIONAL JUSTIFICATIONS

There are numerous areas within exercise physiology that remain ambiguous and lend themselves to further research. As time progresses and new knowledge is acquired from research, man moves a step closer to achieving a state of excellence. Regardless of whether you are a physical education teacher or coach of an Olympic athlete, knowledge of the scientific principles underlying the athletic performance immediately establishes you as a professional person rather than a mere technician (Fox and Mathews, 1981, p.2). It is this statement that forms the basis of this study being justifiably incorporated under the Master of Education program.

There are many questions being asked by scientists around the world on the topic of Branched Chain Amino Acid supplementation as an ergogenic aid. This research attempts to address an area of research which appears to be deficient and in doing so educating these people about this highly contentious topic.

ABSTRACT

Branched-chain amino acid (BCAA) supplementation is becoming more widely used by athletes in an attempt to improve their recovery between training or competition. This study examined the effects of the supplementation on indicators of muscle damage after intense prolonged exercise. Sixteen male subjects were used in the study with eight taking BCAA supplementation and eight acting as the control group. The experimental group were required to take the manufacturer's recommended dose for the 14 days of testing. For the seven days before the exercise test both groups had their blood analysed every second day for creatine kinase and lactate dehydrogenase which have been shown to be accurate indicators of muscle damage. The exercise test was a 120 minute bicycle ride at 70% of their predetermined VO_{2max} . Directly after the exercise and hourly for 4 hours all subjects again had blood samples analysed. The experimental group continued to take supplementation for 7 days after the test and all subjects had their blood analysed every second day. Subjects were also required to keep activity to a minimum and have their diet analysed during the 14 days and manipulated if necessary to ensure that the recommended daily intake of BCAA's were being consumed as part of their normal diet. Results indicated that all subjects' diets did not require manipulation as they all consumed the recommended daily intake of BCAA's in their normal diet. No significant differences were found between any of the two enzyme values in the 7 days prior to the test. There were, however, significant changes in pre-exercise and post-exercise values for LDH at 2 hrs., 3 hrs., 4 hrs., 1 day, 2 days and 4 days after the test ($p < 0.05$). There were also significant changes in pre-exercise and post-exercise values for CK at 4 hrs., 1 day, 2 days and 4 days after the test. The BCAA was shown to significantly ($p < 0.05$) reduce this change in LDH at 3 hrs., 4 hrs. and 1 day and CK at 4 hrs., 1 day and 2 days post exercise. These results indicate that supplementary Branched Chain Amino Acids significantly reduce the levels of the intramuscular enzymes creatine kinase and lactate dehydrogenase after prolonged strenuous exercise. This suggests that the supplementation may reduce muscle damage.

CHAPTER ONE

INTRODUCTION

It is human nature for individuals to bring out the best within themselves. In sport this factor combined with intense competition has seen athletes training harder, longer and more frequently to achieve a winning edge. Burke (1992) stated that athletes are being expected to continually perform at their optimum level during these training sessions, adding that their recovery periods are also being shortened in order to obtain a greater volume of training. The effects on the body are also being compounded by a noticeable increase in the number of times athletes are being enticed to perform at high level competition in many different sports.

Armstrong (1984) concluded that delayed muscle soreness after training or competition is one of the major inhibitors in an athlete's ability to perform at his or her peak in their next exercise bout. The recovery of athletes from delayed muscle soreness between these exercise bouts is a topic which is

gaining more interest by sport science researchers, with many athletes investigating and using a wide range of substances that their manufacturers claim will increase their recovery from muscle soreness after exercise.

One such substance are the branched chain amino acids which, although plentiful in a diet containing animal products, are being claimed by their manufacturer's to give extra benefits when additional dosages are taken. Claims include ;

"Repair of muscle tissue following exercise, eliminating toxins, such as lactic acid and ammonia at the muscle site and stabilizing blood sugar levels"

(Musashi Press Release, 1991)

Slavin (1988) reports that branched chain amino acids (BCAA's) are widely used by various sportspeople in an attempt to aid the recovery process. It is the aim of this study to determine whether the supplementation of BCAA's, into a diet that already has the recommended daily intake effects indicators of muscle damage in a seven day period following intense prolonged exercise.

1.1 Muscular Onset Muscular Soreness (DOMS)

Most athletes, especially those engaged in training or competition for endurance events experience severe muscular discomfort following their workouts. Friden et al. (1981) describes the delayed muscle soreness as having symptoms including stiffness, tenderness and pain especially when the individual is required to make active movements. He states that the signs are firm, tender and weak musculature, and although the pathophysiology behind this condition is not known, he concludes that many theories exist.

In general two types of muscle soreness have been postulated since the early work by Hough (1902). The first is an early and general soreness occurring usually within 4 to 8 hours which disappears rapidly, and secondly, a localized soreness or lameness, also called delayed onset muscular soreness (DOMS), which usually appears 2 to 24 hours after exercise and which may persist for several days. Armstrong (1986) in his review on mechanisms of delayed muscular soreness concludes that, the first type is held to be due to diffusable metabolites, with the second widely believed to be the result of tissue damage, such as torn muscle fibres and/or torn connective tissues. Astrand and Rodahl (1977) supports this by stating that the major symptoms

of delayed muscle soreness usually appear after 12 hours, become more severe during the next day, and gradually fade away, so that the muscles are symptom free after 4 to 6 days. They believe that the pain is probably caused by injuries, especially of the connective tissues, within the muscle and its attachment to the tendon and that secondarily histamine and other substances are produced which may cause oedema and pain.

Friden (1984) states that the degree of the symptoms depends on the athlete's condition prior to the workout and his or her work intensity during the session. These symptoms may seriously affect their ability to train or compete in the near future. Armstrong (1984) concludes that the repair in the damaged tissue results in a stronger muscle, much less susceptible to further injury, even if the subsequent exercise is much more severe.

Various authors have related the perception of delayed muscular soreness to muscle or connective tissue damage brought about by exercise : (Hough, 1902 ; Hill, 1951 ; Asmussen, 1956 and Schwane et al., 1983). Investigators such as Smith, (1968) ; Demos et al., (1974) and Olerud et al., (1976), have used the measurements of intramuscular enzymes, such as creatine kinase (CK) and lactate dehydrogenase (LDH), as the indicators of the muscle damage sustained during an exercise

bout and in the recovery period following. They are considered by Noakes (1987) to be the best objective indicators of muscle injury.

Saks (1978) describes that CK, also known as Creatine Phosphokinase, plays a major role in the intracellular energy transport from mitochondria to myofibrils and other sites of energy utilisation. Due to the existence of the creatine phosphate pathway for energy transport, intracellular creatine phosphate concentration is an important regulatory factor for muscle contraction, which influences the contractile force by determining the rate of regeneration of ATP directly available from myosin ATPase. LDH is shown by Sjodin (1976) to be an enzyme found in most tissues of the body. It catalyses and regulates the formation and turnover of lactate during heavy muscular exercise. In the muscle cell most of the LDH is found in the sarcoplasm.

Before examining the metabolism of the BCAA's, it will be first necessary to understand what occurs at a cellular level after prolonged exercise, so that an understanding can be formulated as to how the supplementation may aid in any processes that occur in the recovery phase. Carlson (1983) has shown that the regeneration of skeletal muscle fibres after damage by exercise can vary in extent and effectiveness. Hodgson (1989) states that the degree to which a damaged fibre can repair itself is clearly

influenced by many factors for example;

"The presence of viable muscle elements from which muscle regeneration may begin either at the extremes of or within a segment of necrotic muscle, the general condition and particularly the endocrine status of the athlete, the integrity of the sarcolemmal sheath, blood supply, tissue oxygenation and the presence of irritants or foreign bodies."

(Hodgson, 1989, p. 121)

He concludes that the single most important factor is the protein synthesis which must occur in the damaged muscles.

If damage to muscle fibres is as common as would be suggested by the prevalence of DOMS, what are the consequences of the injury? First, there is an attenuation of maximal force in the injured muscles, both for voluntary and involuntary contractions. It is this factor which Friden et al. (1983) believes affects the performance of athletes whilst suffering from this condition. He goes on to demonstrate that normal force production returns within 24 hours in human subjects following cycling exercise, and shows it to be longer in eccentric exercise. Before analysing research concentrating on the effects of BCAA supplementation and the indicators of muscle damage, it is first necessary to gain an insight into the mechanisms which are hypothesized as occurring following an intense prolonged exercise bout.

Early investigations of the repair and regeneration of muscle tissue date from the second half of the 18th and the beginning of the 19th century when macroscopic changes were studied in dogs, cats, and rabbits. From these studies, it emerged that the destructive lesions in muscle resulted in fibrous scarring, a conclusion that was generally accepted despite claims that skeletal muscle did in fact possess some power of regeneration.

The early literature has been summarised by Cutner and Landows (1913) in Hodgson (1989). During the second half of the nineteenth century a number of studies were published in which the regenerative capacity of striative muscle was clearly recognised, though different views were taken of the process.

"Allowing for the underdeveloped state of biological technique at the time, the reader today is astonished at the accuracy of many of the observations made and the shrewdness with which they were interpreted."

[Cutner and Landows (1913) in Hodgson (1989),
1913, p. 231]

For example, as early as 1858, Botcher in Hodgson (1989) noted the nuclear proliferation which occurred in the first 24 hours at the margins of muscle wounds in animals. Weber (1863) in Hodgson (1989), concluded that new muscle cells

were derived from old surviving ones, and it is of considerable interest to find that in a later paper there is a suggestion of a budding theory;

"Now and again there can be seen a pale, narrow, faintly striated process of contractile protoplasm issuing from a conically tapering sarcolemmal tube or from its torn off end. This protoplasmic process must be regarded as a new formation, since it is clearly distinct from the old striated fibre substance".

(Weber, 1863 in Hodgson (1989), p. 232)

The similarities of this description with the modern view is striking. Another completely modern idea was brought forward by Ahlfrecht (1868) in Hodgson (1989), when he suggested that the condition of the sarcolemma was of paramount importance for regeneration. Detailed studies were made by Le Gros Clark (1946) and Godman (1957). In the 1960's a great deal of new information emerged on the subject of muscle regeneration, with much of it confirming long held views that sarcolemmal integrity is of paramount importance in determining the outcome of muscle regeneration. More modern work however, has challenged the relative importance of the continuous regeneration (budding theory) from surviving fibre segments as opposed to discontinuous regeneration on the occurrence of mitosis in regenerating muscle cells and the origin of these new cells. Armstrong (1986) believes that

possibly the most significant recent landmark has been the satellite cell theory of muscle regeneration, and the understanding of the role of mitosis in myogenesis and the recognition that regeneration occurs after prolonged exhausting exercise.

A reasonably clear cut chain of events has been defined by Carlson (1983) in the course of degeneration, and these are essentially the same whatever the noxious stimuli inducing them. Both continuous and discontinuous regeneration occur. In the first case, regeneration occurs with the outgrowth of one or more sprouts, or buds from a normal segment to an adjacent necrotic one after the latter has been cleared of necrotic sarcoplasm, myofibrils, etc. by phagocytic cells. The efficiency of this type of regeneration clearly depends on the integrity of the sarcolemmal tube into which the sprouts are growing. In discontinuous regeneration, the process depends on the presence of viable myoblasts, or at least viable myoblast nuclei within the empty sarcolemmal tube, which may act as seeds from which regeneration can occur. In concluding, Carlson (1983) mentions that there is no incompatibility between continuous and discontinuous regeneration and that both probably go on side by side in most cases.

Numerous Studies have demonstrated that training diminishes the muscle fibre injury that results from a given task

(Schwane and Armstrong, 1983 ; Friden et al., 1983 and Jones and Newham, 1985). Why training decreases the injury in the muscles for a given exercise is not known, however Jones and Newham (1985) seem to support the concept that in an untrained muscle, there is a small population of muscle fibres that is susceptible to injury, perhaps through defects in their protein structure. They believe that during training, these susceptible fibres may be damaged at the sites of their structural defects, undergo degeneration and be replaced by fibres that can withstand the tensions developed in the muscle for the given exercise task. Further increases in training intensity or in exertion could reveal another population of susceptible fibres that would be damaged by the elevated tensions.

Carlson and Faulkner (1983) show that damage to skeletal muscle fibres by exhaustive exercise has been documented by myoglobinuria and histological evidence of fibre necrosis. Following exhaustive exercise, increased acid hydrolase activity of muscle homogenates indicates the activation of the lysosomal system of regenerating skeletal muscle. Friden et al. (1983) show that endurance conditioning appears to reduce the amount of activity of the acid hydrolase to a given exercise stress, but highly motivated, well conditioned athletes still demonstrate myoglobinuria following extreme exertion. Exercise enhances recovery of the functional capabilities of regenerating skeletal muscle fibres, but there is little data on which to design

appropriate exercise programs. Questions remain concerning when exercise should start and which intensity and duration are optimal. Much research remains to be done before definitive answers can be formulated.

One possible cause of muscle cell injury is referred to by Armstrong (1984) as "metabolic overload", in which the demand for ATP in the fibre exceeds ATP production. Since muscle cell homeostasis is dependant upon ATP requiring ion pumps, lowered ATP levels in the cell could result in altered ion concentrations in the cell. For example, abnormally elevated Ca^{++} in the muscle fibre is known to trigger a cycle of events in which the mitochondria sequester some of the excess Ca^{++} , which further reduces mitochondrial respiration, which further reduces ATP availability. Thus low ATP levels could initiate a vicious cycle starting with lowered ability to extrude Ca^{++} from the cell via ATP dependant Ca^{++} pumps, and ending in cell death. According to this hypothesis, reduced ATP in the muscle during exercise could initiate this cycle.

A corollary to the metabolic hypothesis is by Abraham (1977), suggesting that high metabolite levels in the muscle during exercise may lead to injury and pain. In particular, lactic acid has been suggested to have a toxic effect on muscles when present in high concentrations, such as during prolonged

intensive exercise (Abraham, 1977, ; Newham et al., 1982 and Armstrong, 1984). This idea has been particularly popular in respect to muscular soreness, and is commonly offered as the explanation for DOMS.

There are several lines of support for the metabolic hypothesis. Perhaps the strongest evidence that exercise-induced muscle fibre injury might have a metabolic basis comes from studies of muscle ischaemia, in which oxygen delivery to the muscles is reduced by occluding their blood flows (Karpati et al., 1974 ; Makitie and Teravainen, 1977). This prevents the cells from maintaining their normal cellular respiration and hence, normal levels of ATP. In these experiments the muscle fibres are injured, and the process and sequence of cell degeneration are very similar to those observed in muscles injured by exercise. DeVries (1960) reported that electromyographic (EMG) activity is elevated in muscles sore from exercise. Referring to the original hypothesis, which suggests that;

"The exercise might initiate a positive feedback creatine kinase cycle where local ischaemia leads to muscle spasm, which in turn causes increased ischaemia and so on, and the progressive increase in ischaemia results in a reduced availability of oxygen in the muscle."

(DeVries, 1960, p. 177)

Saltin and Gollnick (1983) argue against the metabolic explanation. They show that at submaximal exercise such as that encountered in an endurance event, ATP levels in the active muscles appear to be maintained at near resting values. Thus even though ATP use by the myosin cross bridges increases dramatically, the metabolic pathways in the muscle cells apparently are able to keep pace and synthesize ATP rapidly enough to maintain stable ATP concentrations in the active fibres. Schwane et al. (1983) also argue against a metabolic basis for muscle fibre injury during exercise. They concluded that eccentric contractions, which require low energy expenditure, should, on the basis of the metabolic hypothesis cause less muscle damage. However their study showed that a greater degree of muscle damage was found after eccentric exercise.

From his experiments on muscle soreness at the turn of the century, Hough (1902) concluded that delayed soreness results from ruptures of muscle fibres or connective tissue that occur during the exercise. The degree of soreness is related to the peak forces produced by the muscles, but not the extent of acute fatigue of the muscles during the exercise bout. There is now histological and biochemical evidence that structural damage does occur during exercise, particularly eccentric exercise (Friden et al., 1981 ; Newham et al., 1983 and Jones et al., 1986).

1.2 Intramuscular Enzymes

It has been shown by many authors that sustained intense exercise which results in delayed muscular soreness also increases the leakage of substances such as intramuscular enzymes from muscle cells into the circulation in the period following the workout (Hough, 1902 ; Ono, 1953 ; Asinussen, 1956 ; Highman and Altland, 1963 ; Nuttal and Jones, 1968 ; Demos et al., 1974 ; Cerny and Haralambie, 1975 ; King et al., 1976 ; Schumate et al., 1979 ; Roti et al., 1981 ; Kuipers et al., 1982 ; Dickson et al., 1982 ; Melamed et al., 1982 ; Munjal et al., 1983 ; Reinhart et al., 1983 and Armstrong et al., 1983). Thus, if delayed muscular soreness and elevations in serum enzyme activities are induced by exercise, a common cause and effect relationship is conceivable.

The interpretation of the original researchers (Hough 1902 and Asmussen 1956) was that serum enzyme activities increased during and after exercise because of anoxic or hypoxic damage to muscle, particularly to the sarcolemma. It was believed that the rise in serum enzyme activity with exercise was in proportion to the degree of hypoxic damage (Fowler et al., 1968 and Vejjajiva and Teasdale, 1965). Catecholamines are also considered to play a major role as the rise in serum enzyme activity in dogs exposed to altitude was reduced by pre-treatment with adrenergic blocking agents (Highman and Altland, 1960).

Noakes (1987) states that this explanation now seems highly improbable, as it is generally believed that neither hypoxia or anoxia occur during exercise, especially in those activities like ultra marathon running which cause the highest post exercise serum enzyme activities. Rather, it would seem that obvious myofibrillar damage, in particular damage to the sarcomeric z-discs with z-disc streaming, broadening and disruption (Friden et al., 1983), would explain the very large increases in serum enzyme activities measured in athletes after prolonged weight-bearing activities, especially in those athletes who develop marked post exercise muscle soreness.

The smaller increases in serum enzyme activities that occur after exercise of shorter duration, such as running races of up to 21 km., probably indicate changes in muscle membrane permeability without noticeable muscle cell damage. The findings by Haralambie (1973) that the irritability of fatigued muscle is increased as the serum CK and LDH activity is also increased, supports the concept that altered membrane permeability is present in fatigued muscle and is associated with enzyme leakage in blood.

The findings that severe rhabdomyolysis may develop during fasting, or after prolonged exercise, especially after eating a carbohydrate restricted diet, (Bank, 1977 and Carroll et al., 1978) has led to the suggestion that muscle glycogen depletion may

initiate exercise induced rhabdomyolysis. Additional support for this hypothesis is the observation by Wagenmakers et al. (1987) and Wagenmakers et al. (1990) that persons with glycogen phosphorylase deficiency (McArdles syndrome) and in whom glycogenolysis does not occur, develop muscle cramping and rhabdomyolysis when forced to exercise.

Noakes (1987) proposed that the increased mitochondrial mass that develops in skeletal muscle with endurance training may have an additional role to that of increasing skeletal muscle oxidative capacity. It is possible that the increased mitochondrial mass may be better able to prevent oxygen radical damage to muscle. Together with changes to the sarcomeric z-discs this adaptation might explain the effect of training on reducing post-exercise serum enzyme activities. The finding that serum enzyme activities increase markedly after running races longer than 21 km. also supports this hypothesis with severe muscle glycogen depletion occurring in races of 30 km. or more (Sherman et al., 1981). Thus, it is proposed that during exercise, continuing glycogenolysis is necessary for the maintenance of normal cell function, possibly by providing glycolytically produced ATP. This is in line with the belief that glycolytically produced ATP has a unique role in certain specialised cell functions, especially those involving membranes (Bricknell et al., 1981). It was proposed that muscle glycogen depletion disturbs normal sarcolemmal function. This leads to the release of enzymes, as well as the release of the

nephrotoxic substance, myoglobin which results in acute renal failure. However, a direct relationship between muscle glycogen levels after marathon running, and the post-race rise in serum CK activity, was not found in the only study that has investigated this relationship to date (Strachan et al., 1984) .

An alternate hypothesis comes from the observation that children with Duchenne Muscular Dystrophy show a marked increase in serum CK activity in as little as 4 hours after quiet, mild exercise (Florence et al., 1985). There is recent evidence that the capacity to oxidise serum free fatty acids is also impaired in Duchenne Muscular Dystrophy patients (Carroll et al., 1985) leading to increased muscle levels of long chain Acetyl Co-A during exercise (Carroll et al., 1983). It is thought that Acetyl Co-A has a detergent effect on cells and sarcoplasmic membranes, in this way increased intracellular Acetyl Co-A levels could damage the sarcolemma thereby causing myoglobin and cytoplasmic enzymes to appear in the blood. This theory therefore predicts that intracellular Acetyl Co-A levels must increase disproportionately during prolonged exercise, such as running further than 21km.. To explain the lesser rise in serum enzyme activities after cycling then after running, Acetyl Co-A levels would also have to be lower in the muscles of cyclists than in those of runners during exercise. However, at present, this prediction has not been tested.

A third possible explanation by Noakes (1987) is that the elevated rate of oxygen consumption during prolonged exercise leads to the production of various potentially toxic free radical intermediates, which have the capacity for lipid peroxidation, particularly of membrane lipids. Peroxidation damage to cell membranes causes release of intracellular enzymes. The study of Kanter et al. (1986), provides preliminary data relevant to this hypothesis. The serum levels of malondialdehyde correlated with serum enzyme activities prior to a race. Suprisingly however, the correlation was not measured after the race when serum enzyme activities were markedly increased.

Noakes (1987) states that free radical peroxidation cannot be the major factor determining the rise in serum enzyme activities with exercise. If it were, all activities in which the rate of tissue oxygen utilisation, and therefore the rate of free radical production were the same, increases in serum enzyme activities would be equivalent. Thus the free radical theory cannot explain the very much lower serum CK activities measured in cyclists (Noakes 1986) than in runners (Dressendorfer and Wade, 1983) when both perform prolonged exercise for extended periods.

Finally, it is important to appreciate that the elevated serum enzyme activity returns relatively quickly to normal even after ultra endurance exercise (Refsum et al.,1971). Complete recovery from such events, measured as performance in other

running events, may take 3-6 months (Kanter et al.,1986). Thus the increased rate of enzyme leakage from muscle normalises relatively quickly after exercise, whereas full functional recovery takes much longer. The nature of this delayed recovery is presently unknown, but it is clear that it is not reflected by equivalent alterations in serum enzyme activities.

In summary, it would seem that mechanical damage to muscle, explains the rise in serum enzyme activities after prolonged weight bearing exercise. Noakes (1987) suggests that non weight bearing exercises, such as cycle ergometry will cause smaller rises due to changes in membrane permeability, possibly on the basis of muscle glycogen depletion and intracellular Acetyl Co-A accumulation. As this study is observing the effects of prolonged strenuous exercise on serum enzyme activity after BCAA supplementation, it will be necessary to outline their metabolism to identify mechanisms whereby they may effect the cause or causes of the elevated enzyme levels.

1.3 Protein needs of athletes

The protein needs of athletes has been a topic of great debate amongst scientists for many years. Historically, the consensus from the scientific community has been that protein requirements are not significantly increased by heavy exercise (Burke, 1992), and that athletes should look to population RDI's (Recommended

Dietary Intakes) to guide their protein intakes.

Most foods contribute some protein to the diet and the relative importance of foods as a protein source depends on both the amount of food eaten and its protein content. Foods with the highest protein content include meats, eggs, dairy products and legumes. Cereal products such as bread, breakfast cereals and rice have a medium protein content, but still provide a significant source of dietary protein because of their widespread and consistent consumption. Protein quality refers to the ratio of amino acids provided by a protein food. A protein that provides branched chain amino acids in a ratio that matches human requirement is known as having a high quality; such as animal protein, whereas proteins from vegetable sources have a low protein quality.

The body's requirement for protein is actually a requirement for amino acids. Amino acids are the chemicals needed for the synthesis of enzymes, structural proteins, peptide hormones, neurotransmitters, and for the production of energy. Proteins and amino acids are in a dynamic state in the body, being degraded and resynthesized continuously. Some amino acids released during the breakdown of tissue are reutilized, but excess amino acids are oxidized for energy, or stored as fat or carbohydrate (Guyton, 1981).

1.4 Branched Chain Amino Acids

(BCAA)

There are three branched chain amino acids ; leucine, isoleucine and valine and are part of the nine essential amino acids required to be preformed in the diet. During the past years, branched chain amino acids have become the popular ergogenic aid for many different athletes. Promoters claim that the amino acid potions can build muscle, aid fat loss, provide energy and importantly speed up muscle repair (Musashi Press Release, 1991). However Slavin (1988) states that while amino acids are critical for the synthesis of body proteins and other body tissues, it has never been proven that amino acid supplements can benefit athletes who are in good health and are consuming adequate diets. With this in mind, this study also analysed the diets of the subjects involved to ascertain that they were meeting the recommended daily dietary intake of branched chain amino acids. This was carried out with the use of "The first supplement to McCance and Widdowson's, The Composition of Foods" (Paul et al. 1988) and two comparisons. The first by the Australian National Health and Medical Research Council who recommend that a 21 year old male should consume 0.75 g/kg/day of protein and the second by Lemon et al. (1991c) who believes that athletes may need as much as 1.60 g/kg/day. These two different recommendations were used in accordance with Adibi (1976) who stated that the three branched chain amino acids

should make up 40% of the minimal daily protein requirement of man. This calculated to 22.9 grams of BCAA's per day for the Australian National Health and Medical Research Council's recommendation and 47.7 grams for Lemon et al. (1991c).

Adibi (1976) also stated that BCAA's are important not only as substrates for protein synthesis, but also as biochemical regulators or precursors in complex metabolic reactions. Lemon and Nagle (1981) stated that their benefits may be derived from the fact that they are readily transported into the muscle cell and during endurance exercise and recovery are readily oxidised. This he states, is supported by a decrease in their plasma concentration.

Kuipers et al. (1989) have also shown that protein requirements are increased by the repair/recovery needs following muscle damage. It has been suggested by Lemon et al. (1991), that an increased intake of BCAA's could suppress the increased rate of protein degradation, that occurs during and after sustained exercise, as well as increasing protein synthesis in the muscle. Buse and Reid (1975), Fulks et al. (1975) and Li and Jefferson (1978) all reported that BCAA's, particularly leucine, have an anabolic effect on protein metabolism.

Goldberg and Chang (1978) stated that the rate of degradation of the BCAA's in muscle is greater than in the liver,

where these metabolic pathways were first found. Many authors have found that muscle constitutes the primary site in the body for the degradation of these amino acids (Cahill et al.; 1972, Carli et al.; 1992, Wahren et al.; 1973 and Wagenmakers et al.; 1989). Furthermore, unlike most ingested amino acids that are absorbed across the intestine, the BCAA's are not taken up to any great extent by the liver, but instead pass through this organ for use by the peripheral tissues (Felig, 1975). It has also been found that the kidney, adipose tissue and the brain are also very active in the degradation of leucine (Goodman, 1964). Relatively little is known about the physiological significance in these tissues of BCAA metabolism which may serve functions distinct from those of skeletal muscle.

In skeletal and cardiac muscle, the rates of degradation of the BCAA's increase markedly under certain catabolic states, including diabetes and starvation (Buse et al.; 1973, Goldberg and Odessey; 1972), as the overall protein synthesis falls. Buse and Reid (1975) stated that the metabolism of BCAA's in muscle, appears of particular physiological interest, because they have a special ability to promote positive protein balance in this tissue. Goldberg and Chang (1978) stated that the breakdown of BCAA's in muscle, generates amino groups whose accumulation in the organ could be toxic. Unlike liver, skeletal muscle lacks the enzymes to dispose of ammonia as urea.

Alanine production by muscle is described by Felig et al. (1970) as having an important role to play in the maintenance of blood glucose. In the liver, Goldberg and Odessey (1972) stated that alanine represents the most important amino acid precursor for gluconeogenesis. On this basis Felig (1975) proposed the glucose-alanine cycle, in which alanine carries amino groups derived from amino acid metabolism in muscle, and pyruvate to the liver for conversion into urea and glucose. Accordingly, the glucose synthesised by the liver can then be taken up again by the muscle, and be converted back to alanine. A variety of evidence supports the existence of this cycle (Garber et al., 1976, Goldstein and Newsholme; 1976 and Snell and Duff; 1977, Block and Buse; 1990).

Block and Buse (1990) showed how the metabolism of BCAA's differ from that of most other indispensable amino acids in that; 1) the enzymes required for their breakdown are not confined to the liver but are distributed widely throughout the body; 2) the initial reaction in their catabolism is a readily reversible transamination, which occurs mainly in the muscle; 3) the initial transamination reaction appears to be relatively unresponsive to dietary and hormone manipulation; 4) the catalyst for the second reaction, a dehydrogenase which facilitates the irreversible oxidative decarboxylation of branched chain alpha-keto acids formed from the transamination reaction, is considered rate limiting and exists in both active

(dephosphorylated) and inactive (phosphorylated) states. The three BCAA's share common enzymatic steps in their degradation. Specifically, the first three reactions are similar for each of the BCAA's. The initial step in BCAA's catabolism is a readily reversible transamination catalyzed by branched chain amino acid aminotransferase.

Wagenmakers et al. (1989) stated that traditionally, it has been taught that proteins and amino acids do not contribute to energy supply during exercise. This belief was based on the finding that urinary nitrogen excretion did not increase during exercise. Many recent reviews however, suggest that this classical belief may underestimate the role of amino acids as a fuel (Lemon et al.; 1984, Poortmans; 1986 and Viru 1987). Since 1979 several laboratories, applying new and different procedures and techniques, have reported increases in protein and amino acid catabolism during exercise (Decombaz et al.; 1979, Dohm et al.; 1982). The most convincing evidence for increased amino acid oxidation during exercise has been given by Rennie et al. (1981), Lemon et al. (1982), and Wolfe et al.(1982).

Dohm et al. (1987) stated that the rate of degradation of contractile proteins is decreased during exercise, but is increased during the recovery period if the exercise is of high intensity and of long duration. They suggest that the rate of degradation of contractile proteins in muscle, may be influenced by the amount of

BCAA's available at the site, through their various metabolic influences previously discussed .

CHAPTER TWO

REVIEW OF LITERATURE

After an extensive search, it was concluded that no other previous study had examined the effects of BCAA supplementation on indicators of muscle damage. Attempts to reduce the post exercise rise in serum enzyme activities, or reported muscle soreness, have used the techniques to protect against lipid peroxidation by the ingestion of the anti-oxidant, vitamin E (Helgheim et al, 1979, Gillam 1991) or by reducing the inflammatory response by ingesting drugs that inhibit the action of the prostoglandins (Kuipers et al., 1985; Strachan et al.. 1984). None have been successful.

In the past two decades, a review of literature shows that numerous unique characteristics have been attributed to the BCAA's. Research has demonstrated that the BCAA's, and

particularly leucine, stimulate protein synthesis and inhibit protein degradation. Buse and Reid (1974) as well as Fulks et al. (1975) have regarded Leucine as one of the most potent amino acids, especially in the stimulation and regulation of protein synthesis in the skeletal muscle. Garlick and Grant (1988) reported that the BCAA's may achieve this by increasing the activity of muscle to the anabolic actions of insulin.

The importance of BCAA's as metabolic fuels during the stressful situations of increased calorific need (exercise), has also been shown by Adibi et al. (1974) and Ahlborg et al. (1974). It has been observed that the oxidation of BCAA's increases the muscle synthesis and release of alanine. It has been proposed by Odessey et al. (1974), that these amino acids may also regulate the muscle release of ammonia and gluconeogenic precursors for metabolism by the liver.

Elia and Livesey (1983) suggested that the glucose alanine cycle plays an important role during exercise, by accentuating the attention to the BCAA metabolism in muscle tissue. During exercise the outflow of amino acids, particularly alanine increases in muscles. However, the dynamics of the amino acid content of working muscle during, and after prolonged exercise is poorly studied. The total content of amino acids decreased in working muscle and also in the liver during exercise.

Recent studies of supplementation with BCAA's during prolonged endurance exercise have shown mixed results. Blomstrand et al. (1991) reported an improvement in mental function in cross country runners, and an improvement in running performance amongst slower marathon runners, when BCAA's were supplied in a carbohydrate drink during prolonged exercise (30km cross country race or 42 km marathon). The amino acid supplement preserved plasma amino acid levels, compared to the pre-post race drop observed in the placebo group, thus supporting the above hypothesis. Additional evidence by Dohm et al. (1977) suggested that the BCAA's may also have reduced exogenous protein degradation during the marathon race, providing another mechanism of performance enhancement.

Other studies however have failed to support this work. Galliano et al. (1991) added BCAA's to a carbohydrate drink consumed during a prolonged cycle to exhaustion (up to 255 minutes duration), and failed to show any difference in performance between experimental or control conditions. BCAA levels were maintained by the supplement and decreased slightly in the control trial, however, no differences in endocrine response were noted between trials. Vadewalle et al. (1991) supplemented glycogen-depleted subjects with BCAA and failed to find an improvement in time to exhaustion in a subsequent work test, despite a significant increase in plasma BCAA level.

Other recent short reports of amino acid supplementation have reported some improvements in metabolic response to ultraendurance performance (Mitchell et al., 1991 ; Kreider et al., 1991), and an accelerated adaption of muscle buffer capacity during combined anaerobic and aerobic training (Sharp et al. 1991). These studies reported performance enhancement, and the possibility of sparing exogenous protein.

As mentioned previously, no studies were found that looked at BCAA supplementation and its effects on post-exercise recovery and indicators of muscle injury. An interesting study however, was recently carried out on swimmers by Cade et al. (1991). They assessed muscle damage during 6 months of heavy training, using CK and LDH as markers. They reported that the most successful dietary intervention (ie. achieving a lower rise and a more rapid return of plasma levels of these enzymes) was achieved by ingesting a carbohydrate solution during training and consuming a carbohydrate-protein supplement immediately after the session.

Plasma concentrations of BCAA are more prominently affected than those of other amino acids when human subjects are given either a protein, carbohydrate, or fat meal. Adibi and Mercer (1973) stated that the major contributing factor to hyperaminoacidemia after a protein meal or after glucose ingestion is the change in plasma concentrations of BCAA. When a large amount of fat is ingested, the concentrations of BCAA are uniquely

increased in plasma. Observations on plasma concentrations by Galiano et al. (1991) have provided the evidence for alteration of metabolism of BCAA by dietary changes. Furthermore, they raise questions regarding the cellular processes concerned with BCAA metabolism, which may be altered by nutritional status.

Burke (1992) concluded that at the present time, there was insufficient evidence to support the benefits of amino acid supplements that greatly exceed the dietary potential of most athletes. However, there was also insufficient evidence to state conclusively that such supplements do not improve athletic performance. An athlete should take into account all the present evidence, possible side-effects, disadvantages and the knowledge that all other avenues of optimal nutrition, training and lifestyle have been covered. Dietary surveys and anecdotal reports suggest that many athletes do not make such considered decisions. The aim of this study is to determine whether the supplementation of BCAA, into a diet that already has the recommended daily intake, effects the indicators of muscle damage. It is therefore necessary to monitor and manipulate if necessary, the diets of the subjects to ensure that the intake of BCAA through the diet, is in line with recommendations.

PURPOSE OF THE STUDY

The aim of this study was to investigate the effects of BCAA supplementation on indicators of muscle damage, after a prolonged strenuous exercise, a topic which appears yet to be investigated.

DELIMITATIONS OF THE STUDY

The following delimitations of this research were realised by the researcher:

All subjects were unpaid, male, adult, University age and volunteer participants

LIMITATIONS OF THE STUDY

The following limitations in the collection, interpretation and generalisation of the data assumed in this study were realised :

1. The accuracy of all measurements were limited to the regularity of calibration and the consistency of the Kodak Ektachem DT60 analyser and the gas analysis unit.

2. The accuracy of the Sports Tester PE- 3000 Heart Rate telemetry unit.

3. The ability to generalise the results of the study to the characteristics of a broader population, was limited by the physical characteristics of the sample subjects used in the study.

CHAPTER THREE

METHODS

This research project was conducted over a four week period in the Human Performance Laboratory at the University of Tasmania at Launceston, Centre for Physical Education.

SUBJECTS

Sixteen healthy male, Physical Education University students volunteered for this study. They were all required to complete medical and activity questionnaires (Appendix A) which determined that they were free of cardiovascular, orthopaedic and metabolic disorders and that they were all participating in regular physical activity. They were all informed verbally, as well as in writing as to the nature and possible problems associated with the experiment and they all gave written informed consent (Appendices B and C). All experimental procedures were approved by the University of Tasmania's Human Ethics committee. The characteristics of the subjects were (mean \pm SEM): age; 21.3 \pm 1.1 years, height;

178.4 \pm 2.8 cm., mass; 76.3 \pm 1.4 kg., 7 skinfolds, 81.6 \pm 25.9 mm. and maximum oxygen uptake ($\text{VO}_{2\text{ max}}$); 51.6 \pm 8.4 ml. $\text{Kg}^{-1}\text{min}^{-1}$.

EXPERIMENTAL DESIGN

Prior to the start of any testing, the subjects were measured for height, mass and skinfold readings from seven sites (biceps, triceps, subscapular, suprailiac, abdominal, mid-thigh and gastrocnemius) according to protocols suggested by Harrison et al. (1988). Subjects also underwent a maximal Oxygen uptake ($\text{VO}_{2\text{ max}}$) test on a Repco EX 10 bicycle ergometer. This test outlined by Fox and Mathews (1981), also served to familiarise the subjects with the equipment to be used in the exercise test. The incremental bicycle ergometer test to exhaustion, consisted of subjects pedalling at a power output of 150 watts for two minutes. After this initial period, the workload was increased by 50 watts for 2 minutes. This continued until the subjects reached voluntary exhaustion, or they could no longer maintain the pedalling frequency.

Respiratory data were collected during the exercise tests by open circuit spirometry, using an Applied Electrochemistry Oxygen analyser (Model S3-A) and an infrared Heraeus Binos 1 Carbon dioxide analyser. Ventilation was measured via a Yellow Springs Instruments Flow Meter (Model# 2015). Prior to each

of the testing sessions, each analyser was calibrated using three known mixtures of gases; N₂, CO₂, and O₂, and the flow meter volume calibrated using a Hans Rudolph Calibration syringe. During the exercise tests, subjects breathed room air through a mouthpiece connected to a three-way Koegel valve, supported by a lightweight plastic helmet, that was connected via a 3cm diameter low resistance plastic tubing, directly to the mixture chamber. Heart rates were monitored continuously during the test using a Sports Tester PE- 3000 Heart Rate telemetry unit.

The sixteen subjects were randomly divided into two groups of eight. Group A, (the experimental group), were required to take the BCAA supplementation (Musashi - "NI") for the 14 days of their test period. The dosage was that recommended by the manufacturer; 6 grams twice per day, as well as directly before and after the exercise test. Group B, (the control group), were not required to take any supplementation. Both groups underwent the same testing procedures.

All subjects were required to have their diets analysed over their 14 day test period. Each subject was contacted in the evening and asked to relate the foods they consumed that day, along with exercise performed. This allowed ongoing analysis to be carried out. The daily contact with the subjects also enabled those in the experimental group to be reminded to take the supplementation. Diets were analysed and compared with the

National Health and Medical Research Council's (1991) recommendations. Subjects were required to have their diets manipulated by the consumption of extra milk, if necessary, to ensure that the recommended daily intakes of BCAA were being consumed. Appendix D shows the data recording sheet used in the analysis of results for each subject.

One week after the completion of the initial measurements the testing began. For convenience, the testing period was held over four weeks. By staggering the start of each subject's test period, the prolonged exercise test was performed on different days for each subject. Figure 1 shows a schematic illustration of the 14 day protocol for one subject, and the 14 occasions that blood samples were taken. On each occasion the subjects reported to the Human Performance Laboratory, where 2ml. of blood was drawn from the antecubital vein. The protocol was the same for both the experimental and control groups.

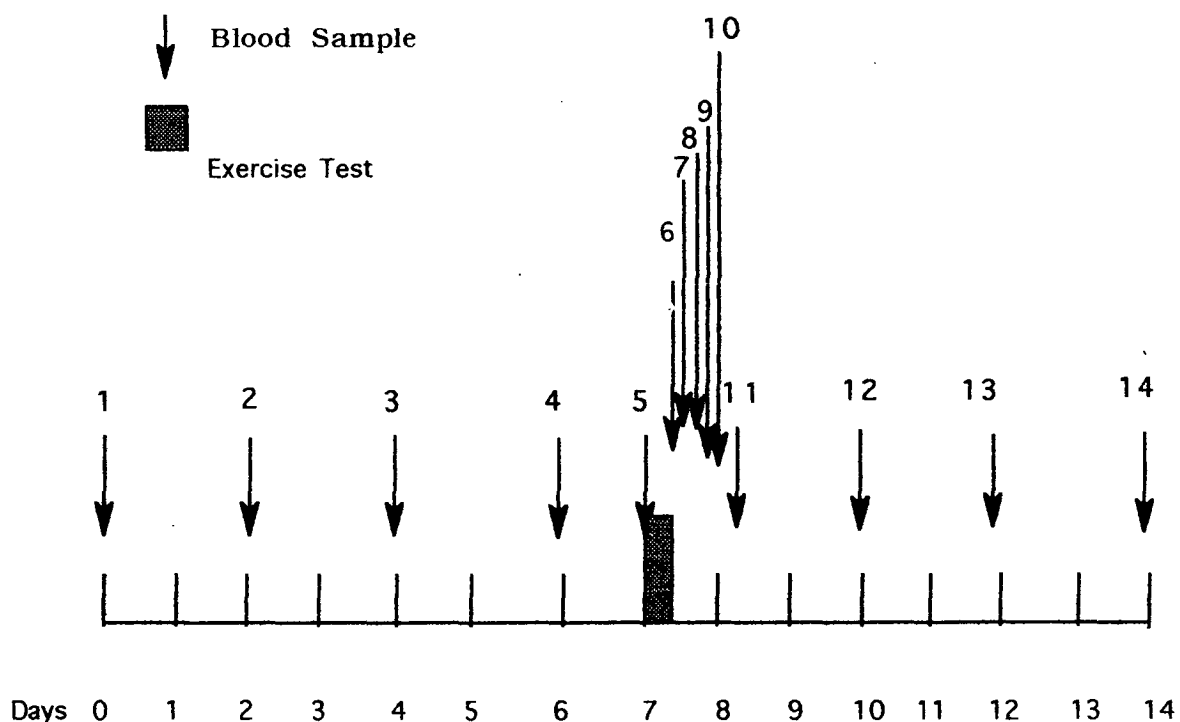


Figure 1

Schematic Illustration of the Studies Protocol

The blood was handled according to protocol suggested by Showell (1992). Each blood sample was placed in test tubes, labelled and centrifuged at 5000 rpm for 10 minutes in a Clements centrifuge to separate the plasma. On removal from the centrifuge, great care was taken not to disturb the constituents. The 10 microlitres (μ l) of serum necessary for the test was then delicately pipetted from the test tube and placed onto a Kodak slide to be analysed for CK and LDH using a Kodak Ektachem DT60 analyser. The methodologies for the analysis of the blood parameters were according to standard Kodak

practices (Kodak, # 352964). The Kodak Ektachem DT60 analyser used in the analysis, was calibrated and checked for quality control using lyophilized material prepared from bovine serum before the first test and at the end of the study. A correlation and a test of significance of difference of means was performed between the first check and the check carried out at the end of the study. There was no significant variation ($p < 0.01$) in either of the two tests between both pre and post quality control tests, indicating the validity, precision and reproducibility of the results using the Kodak Ektachem DT60 analyser.

The exercise test occurred on the morning of day 7 for each subject and required them to cycle on the Repco EX 10 bicycle ergometer for 120 minutes at 70% VO_{2max} . This was calculated from their initial maximal test using a protocol outlined by Fox and Mathews (1981), where heart rate is used to measure percent oxygen uptake. The Sports Tester PE- 3000 Heart Rate telemetry unit was used to monitor the subjects heart rate during the 120 minutes, with subjects advised to increase or decrease workload according to their heart rates.

Table 1 shows the exact times at which the 14 blood samples were taken, and how, that at the completion of the exercise test, and hourly for four hours, blood analyses were carried out (blood tests 6-10). Blood test 11 occurred 24 hours

after the completion of the test, with test's 12-14 two days apart.

Table 1
Time Periods of the 14 blood samples

TEST NO.	DAY
1	0
2	2
3	4
4	6
5	7 (Pre-Ride)
6	7 (Post-Ride)
7	7 (1h. Post)
8	7 (2h. Post)
9	7 (3h. Post)
10	7 (4h. Post)
11	8 (24h. Post)
12	10
13	12
14	14

Data Analysis

Diets were analysed using The first supplement to McCance and Widdowson's "The Composition of Foods" (Paul et al. 1988). An analysis of variance (ANOVA) with repeated measures was used to determine the significance between the CK and LDH results and between the experimental and control groups in the 14 blood tests. A low level of significance of $p < 0.05$ was chosen prior to the start of the experiment.

CHAPTER FOUR

RESULTS

The subjects who were involved in the study were required to have their diets analysed on a daily basis to determine the amount of BCAA that they were consuming in their diet. Figure 2 shows each subject's mean daily intake in milligrams and how they easily exceed the recommended daily intakes as suggested by the Australian National Health and Medical Research Council (NH&MRC), (1991) and Lemon (1991c) in accordance with Abibi (1976). None of the subjects were required to further manipulate their diets.

Level A was calculated by multiplying the mean mass of the subjects (76.3 kg.) by the NH&MRC recommendation (0.75 g/kg/day) by the recommendation by Abibi (1976) on BCAA requirement of 40% of total protein.

(ie. $76.3 \times 0.75 \times 0.40$) = 22.89 grams of BCAA per day

Level B was calculated by multiplying the mean mass of the subjects (76.3 kg.) by the recommendation by Lemon (1991c) (1.60 g/kg/day) by the recommendation by Abibi (1976) on BCAA requirement of 40% of total protein.

(ie. $76.3 \times 1.60 \times 0.40$) = 48.83 grams of BCAA per day

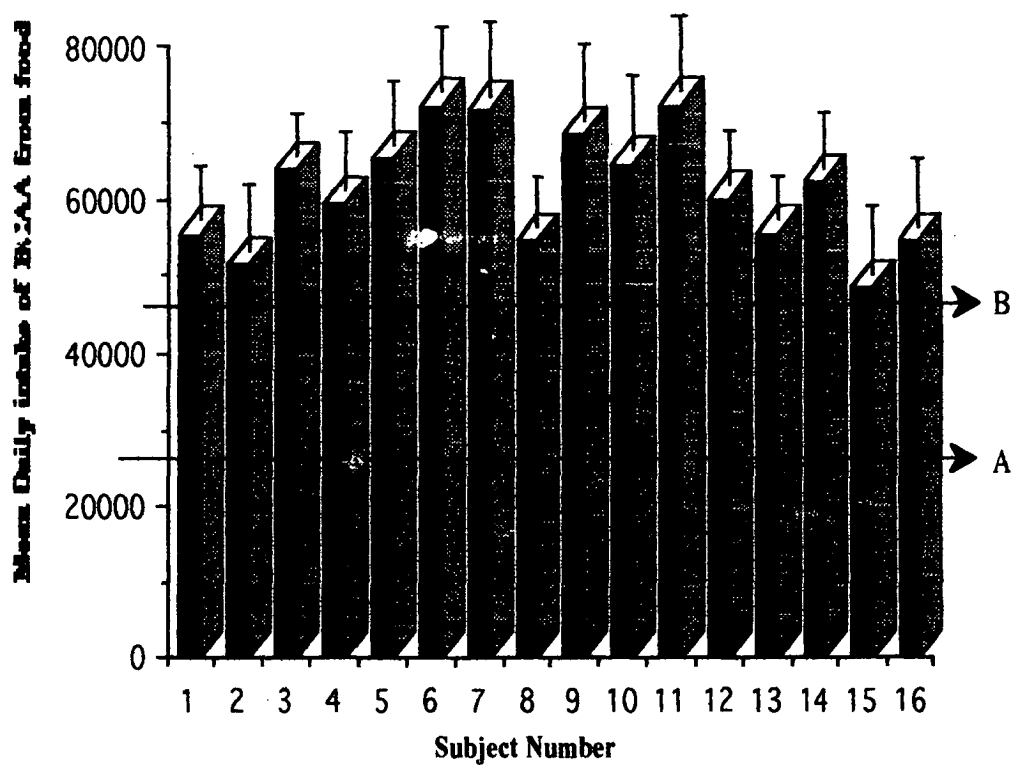


Figure 2.

Bar Graph showing the comparison of the mean daily consumption of Branched Chain Amino Acids with the recommended daily intake by the sixteen subjects.

(S.D. Shown)

A - Australian National Health and Medical Research

Council (NH&MRC), (1991) Recommendation (0.75 g/kg/day).

B - Lemon et al. (1991c)'s recommendation (1.6 g/kg/day)

Table 2 shows the data collected from both groups over the test period, and indicates that the levels of both CK and LDH were elevated significantly higher ($p < 0.05$) after the exercise test in both groups for 5 days after the test. This indicates that serum enzyme leakage had occurred due to the prolonged strenuous exercise. Table 2 also shows that the experimental group was significantly lower ($p < 0.05$) than the control at different times; for CK- 4hr., 24hr., 2 days and 4 days post exercise, and for LDH - 2 hr., 3hr., 4hr., 24hr., 2 days and 4 days post exercise.

Sample	Creatine Kinase (U/L)		Lactate Dehydrogenase (U/L)	
	Exp.	Cont.	Exp.	Cont.
1 (Day 0)	118 \pm 23.6	142 \pm 28.5	565 \pm 47.2	568 \pm 41.7
2 (Day 2)	126 \pm 14.5	130 \pm 14.8	572 \pm 67.1	593 \pm 38.1
3 (Day 4)	104 \pm 19.4	148 \pm 24.0	562 \pm 51.9	601 \pm 63.2
4 (Day 6)	132 \pm 22.5	136 \pm 22.8	596 \pm 49.8	562 \pm 51.9
5 (Pre-Ride)	146 \pm 12.5	151 \pm 23.6	612 \pm 41.7	617 \pm 33.9
6 (Post-Ride)	200 \pm 23.5 ^a	229 \pm 34.4 ^a	771 \pm 65.5 ^a	802 \pm 73.9 ^a
7 (1hr. Post)	257 \pm 37.2 ^a	281 \pm 43.6 ^a	815 \pm 54.7 ^a	843 \pm 48.8 ^a
8 (2hr. Post)	275 \pm 24.8 ^a	307 \pm 37.5 ^a	821 \pm 45.0 ^{ab}	882 \pm 53.1 ^a
9 (3hr. Post)	338 \pm 22.2 ^a	385 \pm 32.1 ^a	823 \pm 42.8 ^{ab}	898 \pm 50.0 ^a
10 (4hr. Post)	362 \pm 23.5 ^{ab}	471 \pm 42.7 ^a	862 \pm 32.9 ^{ab}	927 \pm 65.1 ^a
11 (24hr. Post)	375 \pm 34.4 ^{ab}	525 \pm 51.0 ^a	823 \pm 40.9 ^{ab}	903 \pm 45.9 ^a
12 (Day 10)	331 \pm 30.7 ^{ab}	407 \pm 42.2 ^a	766 \pm 32.8 ^{ab}	822 \pm 61.2 ^a
13 (Day 12)	257 \pm 26.3 ^{ab}	341 \pm 32.5 ^a	680 \pm 45.2 ^{ab}	767 \pm 48.6 ^a
14 (Day 14)	152 \pm 14.2	186 \pm 21.1	588 \pm 34.6	612 \pm 32.0

Table 2

Creatine kinase and lactate dehydrogenase serum levels over a 14 day test period. Means and standard error of the means are indicated.

^a Significantly different from pre-exercise test level (p<0.05)

^b Significantly different from control (p<0.05)

The serum CK changes which occurred in both groups over the 14 day test period are shown in Figure 3. This shows how the initial levels corresponded to the reference range for normal healthy males (55-170 U/L), (Eastman Kodak Company, 1989), indicating very little muscle damage was occurring. There were however, significant differences between the pre-test levels and those taken for the 5 days after the test ($p < 0.05$). This result suggests that muscle damage had occurred because of the exercise test. Figure 3 also shows that the level of serum CK did not rise as high, and the return to resting values was faster, in the experimental group than the control group.

Figure 3
Serum creatine kinase levels of experimental and
control group for the 14 day test period

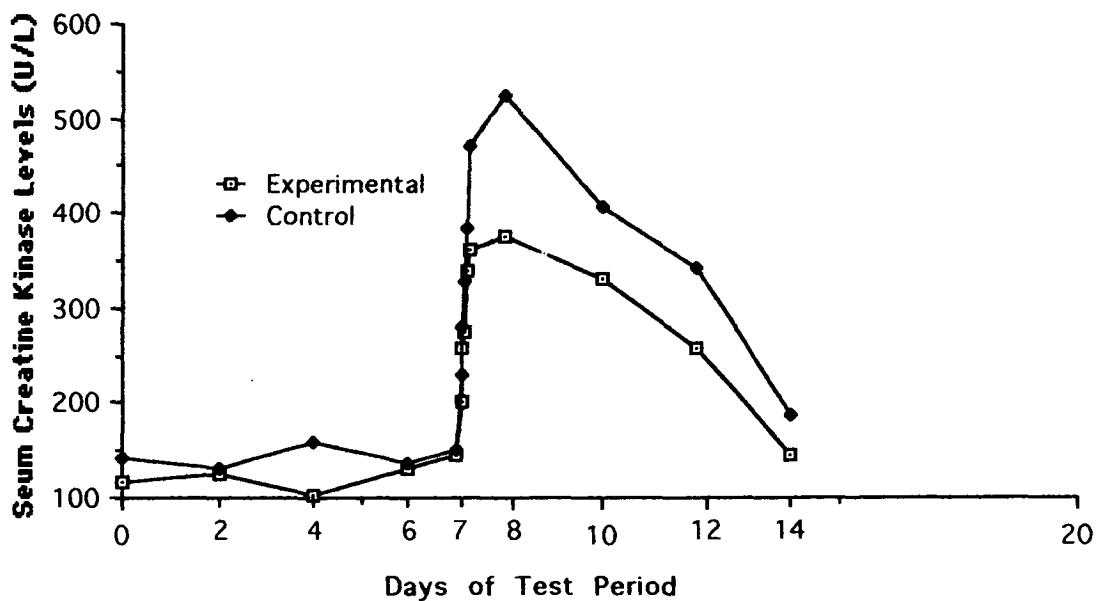


Figure 4 shows the serum LDH changes which occurred in both groups over the 14 day test period. Similar results were found to the CK, with no significant differences between the 5 blood tests taken over the 7 days before the exercise test ($p < 0.05$). These initial levels also indicated that little muscle damage was occurring as they corresponded to the reference range for normal healthy males (313-618 U/L), (Eastman Kodak Company, 1989),. There were however, significant differences between the pre-test levels and those taken for 5 days after the test, ($p < 0.05$) also supporting the theory that muscle damage had occurred because of the exercise test. Figure 4 also shows that the level of serum LDH did not rise as high in the experimental group as in the control group.

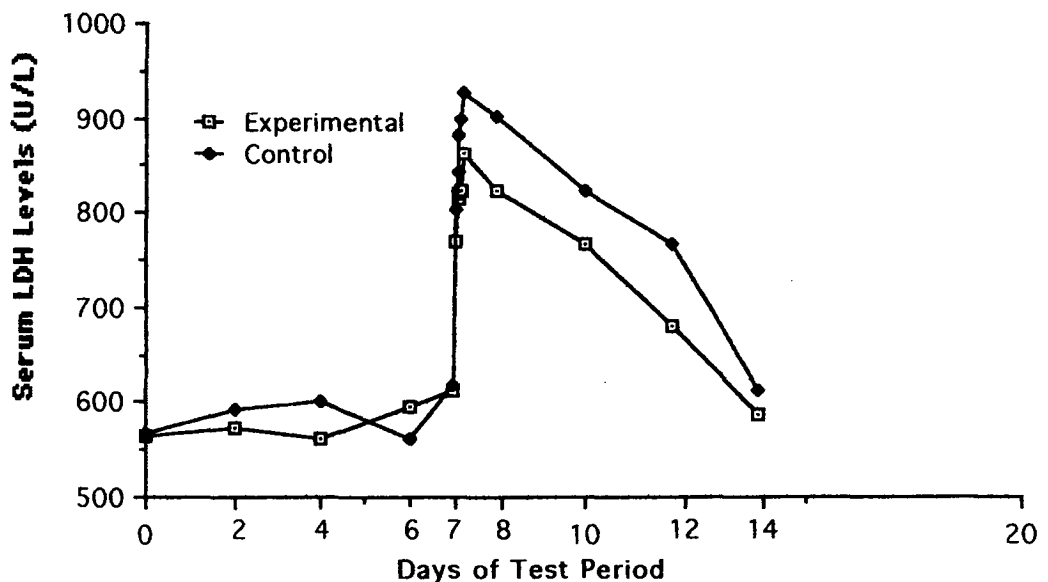


Figure 4
Serum lactate dehydrogenase levels of experimental and control group for the 14 day test period

Figure 3 shows the rise in the serum CK levels over the test period however a closer comparison between the control and experimental groups over the fourteen blood tests can be seen in figure 5. This shows that following the exercise test the CK levels were always lower in the experimental group with significant differences occurring at blood samples 10, 11, 12 and 13 (4hr., 24hr., 3 days and 5 days after the test), ($p < 0.05$).

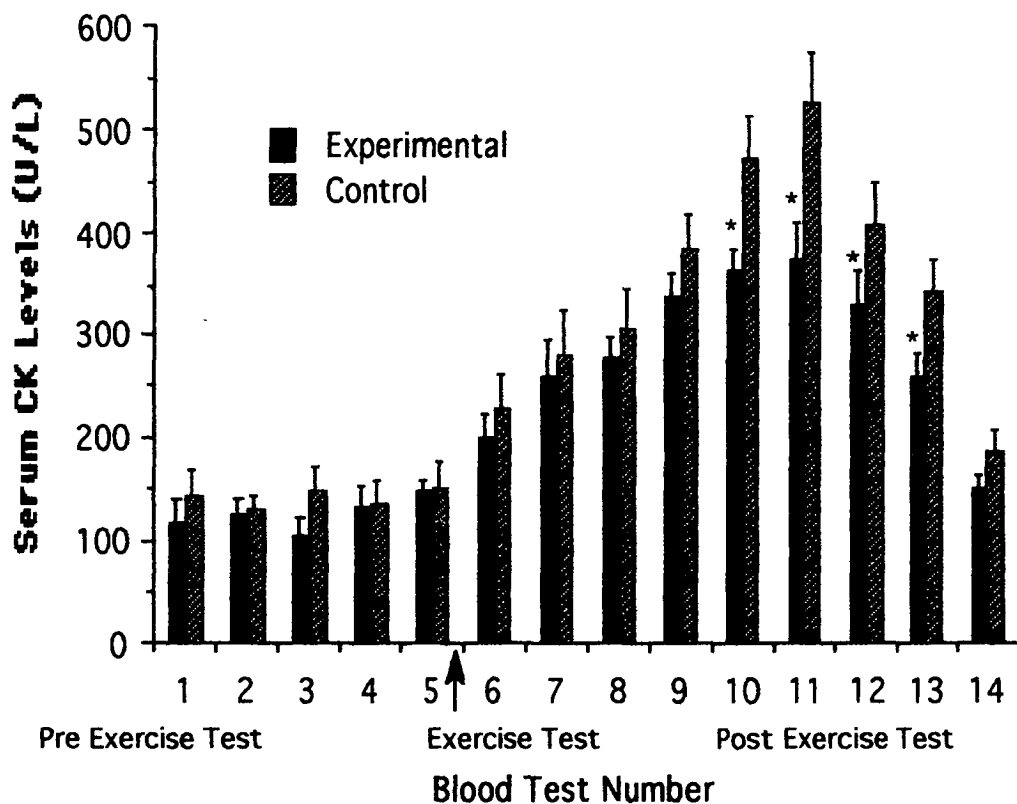


Figure 5

Comparison between the serum creatine kinase levels of the control and experimental groups over the fourteen blood tests (S.D. Shown)

* Significantly lower than Control ($p < 0.05$)

Figure 4 shows the rise in the serum LDH levels over the test period however a closer comparison between the control and experimental groups over the fourteen blood tests can be seen in figure 6. The results show that following the exercise test the LDH levels were always lower in the experimental group with significant differences ($p<0.05$) occurring at blood samples 8, 9, 10, 11, 12 and 13 (2hr., 3 hr., 4hr., 24hr., 3 days and 5 days after the test).

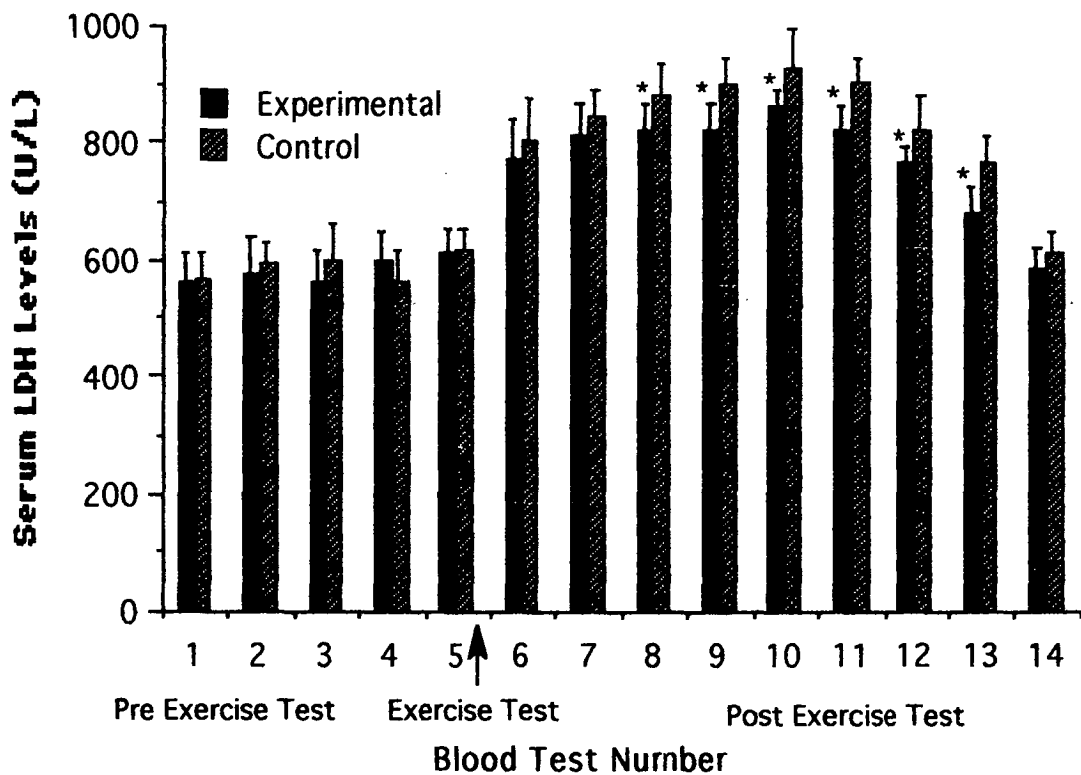


Figure 6

Comparison between the serum lactate dehydrogenase levels of the control and experimental groups over the fourteen blood tests (S.D. Shown)

*Significantly lower than Control ($p<0.05$)

CHAPTER FIVE

DISCUSSION

The cause of muscular soreness which occurs after prolonged strenuous exercise appears to be poorly understood. This delayed pain is believed to be caused by muscle damage and seriously affects the ability of an individual to perform to his or her optimum level. Studies have shown that this muscle damage correlates accurately with the serum levels of the intramuscular enzymes LDH and CK. The results obtained from this study are consistent with work produced by previous researchers that have showed an increase in these levels after prolonged exercise. This study however has demonstrated that the intake of BCAA supplementation into a diet which, already has the recommended dietary intake of these proteins, decreases the levels of these two intramuscular enzymes significantly after a prolonged strenuous exercise test [eg. 24h post exercise test : (CK ; 525 U/L down to 375 U/L) and (LDH ; 903 U/L down to 823 U/L)] . This result would indicate that supplementation of BCAA will decrease muscle damage and perhaps aid an individual's recovery.

The results shown in figure 2 show that all the subjects involved in the study were consuming a diet that exceeded the recommended dietary intake of BCAA as suggested by the Australian National Health and Medical Research Council (NH&MRC); (0.75 g/kg/day) and Lemon (1991c); (1.6 g/kg/day) for protein. Then using Adibi's (1976) adjustment from protein to Branched Chain Amino acids.

In her review on the Amino acid needs of the athlete, Burke (1992) describes how there are a number of methods available to assess protein requirements. Reviews by Lemon (1991a) and Lemon and Proctor (1991) estimate protein requirement based on nitrogen balance studies, and the measurement of the difference between the dietary intake of nitrogen, and the amount secreted or excreted. They state that when the input of amino acids is adequate, the synthesis and degradation of nitrogenous compounds in the body is in balance; nitrogen intake and excretion are equal, and the total body protein content remains stable. If intake is inadequate however, the rate of tissue protein degradation can exceed synthesis and as a result there is an increased loss of body protein and nitrogen excretion exceeds dietary intake. This situation has been termed negative nitrogen balance. Baghurst and Record (1993) conclude that, traditionally two standard deviations in excess of the quantity of protein or individual amino acid necessary to produce nitrogen balance has been set as the

recommended dietary intake (RDI) for the population. Burke (1992) states that the typical Australian diet (with a generous supply of animal food sources) enjoys a high quantity of protein. The subjects involved in this study followed this dietary trend as can be seen from the results in figure 2.

The current information on the protein needs of athletes involved in strenuous prolonged exercise has also been summarised by Lemon (1991a). He suggested that the protein needs of these endurance athletes should be met by intakes higher than those of the Australian National Health and Medical Research Council's recommendations. Figure 2 shows that the subjects involved in this study also surpassed this suggestion for protein in their normal diet.

The results shown in table 2 show that values for the intramuscular enzymes LDH and CK in the seven days prior to the exercise test fell within the normal expected range according to the Eastman Kodak Company (1989) and Griffiths (1966). Griffiths (1966) tested the serum enzyme activity of 353 male adults and concluded that although normal muscular activity in the course of a person's day to day life is a form of prolonged exercise, the extent to which a person's extra activity affects these values is minimal if the activity is not of long duration or high intensity. The subjects were required to keep their activity over the 14 days of testing to a minimal. The enzyme levels in the pre

exercise period were not significantly different ($p < 0.05$), suggesting that the subjects were in a resting state during this time.

The post exercise values for the enzymes are shown in table 2 to have increased significantly for the five days after the exercise test. Figures 3 and 4 give a better indication of the extent of this rise, their peaks and subsequent lowering back to resting values. These results are in accordance with many researchers who have found similar significant increases after long term strenuous exercise; Richterich et al. (1963), Vejjajiva and Teasdale (1965), Griffiths (1965), Griffiths (1966), Ahlborg and Brohult (1966), Nuttall and Jones (1968), Hunter and Critz (1971), Rasch and Schwartz (1972), Buyze and Egberts (1976), Magazanik et al. (1974), King et al. (1976), Heinemann et al. (1978), Kielblock et al. (1979), Seigel et al. (1981), Reinhart et al. (1982), Noakes et al. (1983), Hagerman et al. (1984), Apple et al. (1985) and Kanter et al. (1986). Most of these exercise tests however have involved load bearing activities such as running, which Berg and Haralambie (1978) state; induce a greater enzyme efflux than non weight bearing exercises such as the bicycle ergometer test used in the present study. Research by Schumate et al. (1979) however did measure the effects of a 120 minute bicycle ergometer test (at around 50% VO_{2max} .) on serum CK levels and also found a significant increase in post exercise values.

The time course of the rise in serum CK activity is shown in figure 3. Berg and Haralambie (1978) believe that the rise in values depend on the exercise type, duration, intensity and energy status of the cells. Studies by Fowler et al. (1962), Newham et al. (1983), Nooregard-Hansen (1982) and Farber et al. (1991) all showed a similar rise in CK levels as compared to the present study.

The serum CK activity maximum after exercise as reported by Nuttal and Jones (1968) and Meltzer et al. (1970) is also in accordance with the present findings, with an overall maximal increase of around 400% 24 hours after the exercise test. Nuttal and Jones (1968) speculated that the delay in the maximal elevation of serum CK activity may be caused by an increasing membrane permeability. They hypothesise that following the exercise bout membrane integrity is maintained preventing immediate loss of enzymes into the circulation. Evidence of this is supported by the time sequence rise of serum enzymes following coronary thrombosis, where an immediate increase is uncommon and the peak rise occurs many hours following acute anoxia of the cardiac muscle cells.

Figure 4 indicates that the maximum increase in LDH occurs at 4 hours post exercise where it rises by around 20%. This finding correlates with data published by Sjodin (1976), Sanders and Bloor (1975) and Tidus and Ianuzzo (1983) where

LDH activity reached a peak at 4- 8 hours post exercise. The explanation of the relatively smaller increase, as compared to CK, is given by Bratton et al. (1962) as being due to its larger molecular weight, and subsequent lower ability to escape from the muscle cell.

The CK and LDH levels are shown in figures 3 and 4 to return to pre exercise levels by the seventh day following the exercise test. Berg and Haralambie (1978) believe that the return to resting levels is an indication of the muscle decreasing its energetic demands. They describe these demands as an energy deficit, where there is insufficient energy production to meet requirements such as repair of tissue destruction. As muscle regeneration is completed the energy requirements are returned to normal. From the results in table 2 and figures 3 and 4 it can be concluded that the exercise test had the desired effect of significantly increasing the two intramuscular enzyme levels. Throughout the literature, it is stated that the significant rise of these enzymes indicates that muscle damage has occurred.

Figures 5 and 6 show the comparison between the control and experimental groups over the 14 blood tests. It is interesting to note that the levels in the control group are higher in every blood sample with both enzymes. The enzyme CK is shown in figure 5 to have significantly lower levels

($p < 0.05$) in the experimental group at blood samples 10, 11, 12 and 13 (4hr., 24 hrs., 3 days and 5 days after the exercise test). Figure 6 shows LDH to have significantly lower levels ($p < 0.05$) in the experimental group at blood samples 8, 9, 10, 11, 12 and 13 (2 hr., 3hr., 4hr., 24 hrs., 3 days and 5 days after the exercise test).

The question which must now be answered is how has the BCAA supplementation significantly affected the indicators of muscle damage?. As previously stated, BCAA are used directly as oxidizable foods during exercise, as well as being recognised as major gluconeogenic precursors. Brooks (1987) states that via this mechanism, the amino acids play crucial roles in providing the carbon sources for maintaining blood glucose during exercise and recovery, and most importantly, glycogen restitution after endurance exercise. Noakes (1987), in explaining muscular damage after prolonged exercise, concludes that the rise in intramuscular serum enzymes may be due to changes in membrane permeability due to glycogen depletion. Therefore it is suggested that the BCAA supplementation may increase glycogen restitution which has a positive effect on inhibiting muscle damage due to prolonged exercise.

This hypothesis is supported by the work of Wagenmakers et al. (1987) where patients with McArdle's disease (cannot use muscle glycogen as an energy source during exercise) have

reported an improvement in muscle function when taking BCAA supplementation. The underlying mechanism of the BCAA oxidation is controlled by the limiting enzyme branched chain keto acid dehydrogenase. Lemon and Chaney (1988) believe that supplementation of BCAA further activates this enzyme which in turn increases BCAA oxidation. The increased oxidation facilitates the regeneration of the damaged skeletal muscle by supplying the energy for resynthesis of glycogen and repair of the injured tissues. Conversely, low protein intake (decreased dietary BCAA) decreases BCAA oxidation, which may limit the repair of damaged muscle (Lemon et al., 1991a).

There are other possible explanations of the mechanism of BCAA supplementation on the indicators of muscle damage. Felig and Wahren (1971) observed that alanine is released by exercising muscle and they proposed the 'glucose-alanine cycle'. Odessey et al. (1974) found that alanine release was increased when additional leucine was provided, thus suggesting that this BCAA contributes a large portion to the transamination of pyruvate so that the glucose-alanine cycle can function. Davies et al. (1980) found that the maintenance of this cycle is critical for muscle regeneration after exercise. They also found that the increased BCAA oxidation persists for some time after a strenuous exercise bout. These studies give further hypothetical mechanisms which may explain how the levels of the muscle damage indicators CK and LDH were lower in those subjects who

had taken BCAA supplementation.

A final explanation comes from the work of Buse and Reid (1975), Fulks et al. (1975), Li and Jefferson (1978) and Hodgson (1989) who have all reported that BCAA, particularly leucine, have an anabolic effect on protein metabolism. Lemon (1991a) suggests that an increased intake of BCAA could suppress the increased rate of protein degradation that occurs during and after sustained intensive exercise, as well as protein synthesis in the muscle. It is this increased negative protein balance which many researchers (Goldberg and Chang, 1978 ; Goodman, 1985 ; Hodgson, 1989 ; Buse and Reid, 1975 ; Blomstrand et al. 1991) have put forward as a theory of delayed muscle soreness due to muscle damage. It is evident however that further work needs to be carried out at the cellular level to determine the exact mechanism or mechanisms that have affected the muscle damage indicators.

A further area of concern which must be addressed, is the question of why, if the diets already contained an abundance of BCAA (up to 40 grams higher per day than the intake recommended by the Australian National Health and Medical Research Council), did supplementation of an extra 12 grams per day have such a significant effect ? The answer to this may lie in the work carried out on the regulation of the limiting enzyme branched chain keto acid dehydrogenase by Paxton and

Harris (1984) and Aftring et al. (1986). They found that the enzyme catalyzes the oxidative decarboxylation of the BCAA and Elia and Livesey (1983) determined that this process is increased when a diet high in protein is consumed. This would indicate that the consumption of the amino acid supplementation would also have a positive effect on oxidation and its rate may be increased markedly due to the greater concentration in the supplements. Again, further research needs to be conducted at the cellular level to determine if this hypothesis is valid.

Burke (1992) outlines why high protein diets have been discouraged by most nutritionists in previous years. She explains how protein metabolism increases the work of the kidneys and causes additional urinary water loss for the disposal of urea, concluding that the dehydrating effects of exercise and the self induced dehydration may pose some threats. Another risk associated with a high protein diet is a tendency to increase calcium loss (Barr, 1987), however these risks can be overcome by following other goals of healthy sports nutrition.

Many athletes also consume high fat intakes in conjunction with their high protein foods. These athletes may overlook their carbohydrate needs. In a study by Cade et al. (1991) which assessed muscle damage during 6 months of heavy training using CK and LDH as markers they reported that the most successful

dietary intervention (ie. achieving a lower rise and a more rapid return of plasma levels of these enzymes) was achieved by ingesting a carbohydrate solution during training and consuming a carbohydrate-protein supplement after the session.

More caution is advised with respect to the use of large doses of individual amino acids, as Lemon (1991b) states that; these practices remain largely untested. It is known that large intakes of some amino acids can interfere with absorption and lead to metabolic imbalances (Harper et al., 1970). Lemon (1991b) points out that some studies of amino acids have reported gastrointestinal side effects, and that some amino acids are very toxic at least in animals.

Another possible disadvantage associated with the use of BCAA supplementation is the high cost of the products. A gram for gram comparison by Burke (1992) of the cost of protein and individual amino acids shows that the commercial supplements are around twice as expensive as their food source. In addition the food source may provide additional value by supplying other important nutrients for this price. There are many anecdotes that athletes spend enormous amounts of money on these ergogenic aids.

In summary, the results of this study have demonstrated that the addition of BCAA supplementation into a diet already

high in protein, has had a significant effect on decreasing the intramuscular enzymes CK and LDH [eg. 24h post exercise test : (CK ; 525 U/L down to 375 U/L) and (LDH ; 903 U/L down to 823 U/L)]. These enzymes are shown in this experiment, as well as many previous studies, to be indicators of muscle damage. Therefore the present study concludes that the practice of using BCAA supplementation with healthy young males will decrease the levels of LDH and CK and therefore muscle damage after strenuous prolonged exercise, thus aiding in the recovery process. The response of highly trained athletes to BCAA supplementation may well vary from this and would be an interesting area for further study.

APPENDIX A

MEDICAL AND ACTIVITY QUESTIONNAIRE

MEDICAL AND ACTIVITY QUESTIONNAIRE

Name _____

Medical

1. Has your doctor ever said you have heart trouble ? _____
2. Do you frequently have pains in your heart or chest ? _____
3. Do you often feel faint or have spells of severe dizziness ? _____
4. Has your doctor ever said that your blood pressure was too high? _____
5. Has your doctor ever said that you have a bone or joint problem such as arthritis that has been aggravated by exercise, or might be made worse with exercise ? _____
6. Is there a good physical reason not mentioned here why you should not perform the leg extension exercise at maximal exertion for 80 seconds ?

7. Do you take any prescribed medications ? If yes, please list.

8. When was your last medical checkup ? _____
9. Please name your regular doctor _____

Activity

10. What is your level of physical activity per week (Describe) ? _____

APPENDIX B

HUMAN SUBJECT

INFORMED CONSENT FORM A

(Experimental Group)

**University of Tasmania- Launceston Campus
Department of Physical Education
Human Performance Laboratory**

**Human Subjects Informed Consent Form
Experimental Group**

Jeff Coombes - Principal Investigator

1. Purpose : The aim of this research is to determine whether Branched Chain Amino Acid supplementation effects biochemical indicators of muscle damage after prolonged strenuous exercise.

2. Procedures : You will be required to undergo the following procedures;

1. a) Complete a confidential medical and activity questionnaire
- b) Perform a VO_{2max} test which is an incremental bicycle test, where the resistance is increased slowly over a period of time (usually 8-10 minutes, and you will be required to breathe through a mouthpiece connected by tubes to a gas analyser
- c) Undergo skinfold, height and weight measurements
then on another occasion
2. a) Have 2ml. of blood taken from a forearm vein
- b) Prepare and drink each day (for 14 days) an amino acid mixture (supplied)
then on days 2,4 and 6
3. Have 2ml. of blood taken from a forearm vein
then on day 7
4. a) Have 2ml. of blood taken from a forearm vein
- b) Perform a 120 minute bicycle ergometer ride at 70% of VO_{2max} .
- c) Have 2ml. of blood taken from a forearm vein
then hourly for 4 hours
5. Have 2ml. of blood taken from a forearm vein
then 24 hours later
6. Have 2ml. of blood taken from a forearm vein
then every second day for 6 days
7. Have 2ml. of blood taken from a forearm vein
every evening for the 14 days
8. Answer phone call from researcher about daily food intake

This is a total of 14 Blood Tests

- 3. Discomforts and Possible Hazards :**
- The VO_{2max} requires exerc exhaustion
 - The 120 minute ergometer ride may be of some discomfort
 - The blood tests are relatively painless
 - The taste of the amino acid drink is generally acceptable. however slightly unpalatable

4. Time needed : - VO₂max. test and measurements - 20 mins.

- The drink should only take 2 minutes to prepare and drink each day
- Each visit to the lab. for blood sampling - 5 minutes
- Exercise test - 2.5 hours
- Phone call to be answered each evening - 1 minute

5. Potential Benefits : Research on this topic is being sought after by athletic bodies world - wide

6. Consent : The investigator, Jeff Coombes will answer any questions about the experimental procedures or the results of the tests.

I [the participant] have read the information above and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw at any time and that my future care will not be prejudiced in any way by my refusal to participate.

I agree that research data gathered for this study may be published provided my name is not used.

Participant or authorised Representative

Date

Investigator

Date

APPENDIX C

HUMAN SUBJECT

INFORMED CONSENT FORM B

(Control Group)

University of Tasmania- Launceston Campus
Department of Physical Education
Human Performance Laboratory

Human Subjects Informed Consent Form
Control Group

Jeff Coombes - Principal Investigator

1. Purpose : The aim of this research is to determine whether Branched Chain Amino Acid supplementation effects biochemical indicators of muscle damage after prolonged strenuous exercise.

2. Procedures : You will be required to undergo the following procedures;
1. a) Complete a confidential medical and activity questionnaire

b) Perform a VO_{2max} . test which is an incremental bicycle test, where the resistance is increased slowly over a period of time (usually 8-10 minutes, and you will be required to breathe through a mouthpiece connected by tubes to a gas analyser

c) Undergo skinfold, height and weight measurements
then on another occasion

2. a) Have 2ml. of blood taken from a forearm vein
then on days 2,4 and 6

3. Have 2ml. of blood taken from a forearm vein
then on day 7

4. a) Have 2ml. of blood taken from a forearm vein
b) Perform a 120 minute bicycle ergometer ride at 70% of VO_{2max} .

c) Have 2ml. of blood taken from a forearm vein
then hourly for 4 hours

5. Have 2ml. of blood taken from a forearm vein
then 24 hours later

6. Have 2ml. of blood taken from a forearm vein
then every second day for 6 days

7. Have 2ml. of blood taken from a forearm vein
every evening for the 14 days

8. Answer phone call from researcher about daily food intake

This is a total of 14 Blood Tests

3. Discomforts and Possible Hazards :

- The VO_{2max} . requires exercise exhaustion
- The 120 minute ergometer ride may be of some discomfort
- The blood tests are relatively painless

- 4. Time needed :** - VO₂max. test and measurements - 20 mins.
- Each visit to the lab. for blood sampling - 5 minutes
- Exercise test - 2.5 hours
- Phone call to be answered each evening - 1 minute

5. Potential Benefits : Research on this topic is being sought after by athletic bodies world - wide

6. Consent : The investigator, Jeff Coombes will answer any questions about the experimental procedures or the results of the tests.

I [the participant] have read the information above and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw at any time and that my future care will not be prejudiced in any way by my refusal to participate.

I agree that research data gathered for this study may be published provided my name is not used.

Participant or authorised Representative

Date

Investigator

Date

APPENDIX D

Data Recording Sheet

NAME..... PHONE..... GROUP

AGE..... HEIGHT WEIGHT.....

SKINFOLDS B..... T..... SS..... SI.....

A..... Q..... C..... TOTAL

VO₂max
TEST

Time	Load	Heart Rate
0		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		

Time	Load	Heart Rate
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		

VO₂max LOAD HEART RATE

2 HOUR RIDE LOAD HEART RATE

INDICATORS ;

TEST NO.	DAY	CK	LDH
1	0		
2	2		
3	4		
4	6		
5	7 (Pre-Ride)		
6	7 (Post-Ride)		
7	7 (1hr. Post)		
8	7 (2hr. Post)		
9	7 (3hr. Post)		
10	7 (4hr. Post)		
11	8 (24hr. Post)		
12	10		
13	12		
14	14		

DIETARY/ EXERCISE ANALYSES ;

DAY	FOOD/EXERCISE	AMINO ACID CONTENT
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
TOTAL		

BIBLIOGRAPHY

Abraham W.M., Factors in Delayed Muscle Soreness, Medicine and Science in Sports and Exercise, 9, 1 : 11-20, 1977.

Adibi S.A. and Mercer D.W., Protein Digestion in Human Intestine as Reflected in Luminal Mucosal, and Plasma Amino Acid Concentrations after Meals, Journal of Clinical Investigation, 52 : 1586-1594, 1973.

Adibi S.A., Krzysik B.A., Morse E.L., Amin P.M., and Allen E.R., Oxidative energy metabolism in the skeletal muscle : Biochemical and ultrastructural evidence for adaptive changes, Journal of Laboratory and Clinical Medicine, 83 : 548-562, 1974.

Adibi S.A., Metabolism of Branched Chain Amino Acids in Altered Nutrition, Metabolism, 25, 11 : 1287-1302, 1976.

Aftring R.P., Block K.P. and Buse M.G., Leucine and Isoleucine activate Skeletal Muscle Branched Chain Alpha Keto Acid Dehydrogenase in Vivo, American Journal of Physiology, 250 : E599-E604, 1986.

Ahlborg B. and Brohult J., Metabolic Changes After Exercise, The Lancet, 1 : 1272, 1966.

Ahlborg G., Felig P., Hagenfeldt L. Hendler R., and Wahren J., Substrate turnover during prolonged exercise in man, Journal of Clinical Investigation, 53 : 1080-1090, 1974.

Apple F.S. and McGue M.K., Serum Enzyme Changes During Marathon Training, American Journal of Clinical Pathology, 79 : 716-719, 1983.

Apple F.S., Rogers M.A., Sherman W.M., Costill D.L., Hagerman F.C. and Ivy J.L., Profile of Creatine Kinase Isoenzymes in Skeletal Muscles of Marathon Runners, Clinical Chemistry, 30, 3 : 413-416, 1984.

Apple F.S., Rogers M.A., Casal D.C., Sherman W.M., and Ivy J.L., Creatine Kinase-MB Isoenzyme adaptations in Stressed Human Skeletal Muscle of Marathon Runners, Journal of Applied Physiology, 59,1 : 149-153, 1985.

Apple F.S., Rogers M.A. and Ivy J.L., Creatine Kinase Isoenzyme MM Variants in Skeletal Muscle and Plasma from Marathon Runners, Clinical Chemistry, 32,1 : 41-44, 1986.

Armstrong R.B., Ogilvie R.W. and Schwane J.A., Eccentric Exercise Induced Injury to Rat Skeletal Muscle, Journal of Applied Physiology, 54 : 80-93, 1983.

Armstrong R.B., Mechanisms of Exercised-Induced Delayed Onset Muscle Soreness : A Brief Review, Medicine and Science in Sports and Exercise, 16(6), : 529-538, 1984.

Armstrong R.B., Muscle Damage and Endurance Events, Sports Medicine, 3 : 370-381, 1986.

Armstrong R.B., Mechanisms of Exercise-Induced Delayed Onset Muscular Soreness : A Brief Review, Medicine and Science in Sports and Exercise, 16, 6 : 529-538, 1990.

Asmussen E., Observations on Experimental Muscular Soreness, Acta Rheum. Scand., 2 : 109-116, 1956.

Astrand P.O. and Rodahl K., Textbook of work Physiology, McGraw Hill, New York, 1977.

Baghurst K.I. and Record S.J., Intake and Sources, in Selected Australian Subpopulations, of Dietary Constituents Implicated in the Etiology of Disease, Journal of Food and Nutrition, 40 : 1-15, 1983.

Ball K.P., Kirby B.J. and Bogen C., Serum Creatine Kinase and Physical Exercise, British Medical Journal, 1 : 1653-1654, 1965.

Bank W.K., Myoglobinuria in Marathon Runners : Possible Relationship to Carbohydrate and Lipid Metabolism, The Marathon, Milvy P. (ed.), New York, Academy of Sciences : 942-948, 1977.

Bark S., Bergstrom K., Eriksson S., Henriksson J. and Lindberg K., Serum Enzyme CK-B Rise Following Exhaustive Physical Exercise, American Heart Journal, 102 : 1079, 1981.

Barr S.I., Women Nutrition and Exercise : A Review of Athletes Intake and a Discussion of Energy Balance in Active Women, Progress in Food and Nutrition Science, 11 : 307-361, 1987.

Berg A. and Haralambie G., Changes in Serum Creatine Kinase and Hexose Phosphate Isomerase Activity with Exercise Duration, European Journal of Applied Physiology, 39 : 191-201, 1978.

Berne R.M. and Levy M.N., Physiology, C.V. Mosby Company, St. Louis, 1988.

Block K.P. and Buse M.G., Glucocorticoid Regulation of Muscle Branched Chain Amino Acid Metabolism, Medicine and Science in Sports and Exercise, 22, 3 : 316-324, 1990.

Blomstrand E., Perrett D., Parry-Billings M. and Newsholme E.A., Effect of Sustained Exercise on Plasma Amino Acid Concentrations and on 5-Hydroxytryptamine Metabolism in Six Different Brain Regions in the Rat, Acta. Physiol. Scand., 136 : 473-481, 1989.

Blomstrand E., Hassmen P., Ekblom B. and Newsholme E.A., Administration of Branched Chain Amino Acids During Sustained Exercise - Effects on Performance and on Plasma Concentration of Some Amino Acids, European Journal of Applied Physiology, 63 : 83-88, 1991.

Bratton R.D., Chowdhury S.R., Fowler W.M., Gardner G.W. and Pearson C.M., Effect of Exercise on Serum Enzyme Levels in Untrained Males, The Research Quarterly, 33, 2 : 182-193, 1962.

Bricknell O.L., Daries P.S. and Ople L.H., A Relationship between Adenosine Triphosphate Glycolysis and Ischaemic Contracture in the Isolated Rat Heart, Journal of Molecular and Cellular Cardiology, 13 : 941-945, 1981.

Brooks G.A., Amino Acid and Protein Metabolism During Exercise and Recovery, Medicine and Science in Sports and Exercise, 19, 5 (Suppl.) : S150-S156, 1987.

Burke L.M., Protein and Amino Acid Needs of the Athlete, State of the Art Review, Australian Sports Commission, 28, April 1992.

Burke L.M. and Read R.S.D., Dietary supplements in sport, Sports Medicine, 15 (1): 43-65, 1993.

Buse M.G., Herlong H.F. and Weigand D.A., The Regulation of Protein Synthesis in Muscle, Endocrinology, 88 : 1166-1175, 1973.

Buse, M.G. and Reid, S.S., Leucine : A Possible Regulator of Protein synthesis in Muscles, Journal of Clinical Investigation, 53 :13a-14a, 1974.

Buse, M.G. and Reid, S.S., Leucine : A Possible Regulator of Protein Turnover in Muscle, Journal of Clinical Investigation, 56 : 1250-1261, 1975.

Buyze M.G. and Egberts P.F.C., Serum Enzyne Activity and Physical Condition, Journal of Sports Medicine, 16 : 155-164, 1976.

Cade J.R., Reese R.H., Privette R.M., Hommen N.M., Rogers J.L. and Fregly M.J., Dietary Intervention and Training in Swimmers, European Journal of Applied Physiology, 63 : 210-215, 1991.

Cahill G.F., Aoki T.T. and Marliss E.B., Handbook of Physiology Endocrinology : Volume 1, Endocrine Pancreas, Steiner D.F. and Freinke N. (eds.), American Physiological Society, Washington, 1972.

Carli G., Bonafazi M., Lodi L., Lupo C., Martelli G and Viti A., Changes in the Exercise-Induced Hormone Response to Branched Chain Amino Acid Administration, European Journal of Applied Physiology, 64 : 272-277, 1992.

Carlson B.M. and Faulkner J.A., The Regeneration of Skeletal Muscle Fibers Following Injury : A Review, Medicine and Science in Sports and Exercise, 15, 3 : 187-198, 1983.

Carlson B.M., The Regeneration of Skeletal Muscle - A Review, American Journal of Anatomy, 137 : 119-150, 1983.

Carmer, S.G. and Swanson, M.R., An evaluation of ten pairwise multiple comparison procedures by Monte Carlo methods. Journal of the American Statistical Association, Vol. 68, pp. 66-74, 1973.

Carroll J.E., Brooke M.H., DeVivo D.C., Kaiser K.K. and Hagberg J.M., Biochemical and Physiologic Consequences of Carnitine Palmityltransferase Deficiency, Muscle and Nerve, 1 : 103-110, 1978.

Carroll J.E., Villadiego A. and Brooke M.H., Increased long-chain Actyl Co-A in Duschennes Muscular Dystrophy, Neurology, 33 : 1507-1510, 1983.

Carroll J.E., Norris B.J. and Brooke M.H., Defective (U-14C) palmitic acid oxidation in Duschennes Muscular Dystrophy Neurology, 35 : 96-97, 1985.

Cerny F.J. and Haralambie G., Exercise Intensity vs. Duration as Related to Loss of Muscle Enzymes into Serum, Medicine and Science in Sports and Exercise, 7, 65 (abstract), 1975.

Cohen, J. and Cohen, P., Applied multiple regression/correlation analysis for the behavioural sciences, Lawrence Erlbaum Associates, New Jersey, 1975.

Costill D.L., Fink W.J. and Pollock M.L., Muscle Fiber Composition and Enzyme Activities of Elite Distance Runners, Medicine and Science in Sports and Exercise, 8, 2 : 96-100, 1976.

Davies C.T.M., Edwards R.H.T., Halliday D., Krywawych S., Millward D.J. and Rennie M.J., Increased Branched Chain Amino Acid Oxidation as a Result of Exercise in Man, Proceedings of the Physiological Society, 305 : 89P, 1980.

Decombaz J., Reinhardt P., Anantharaman K., von Glutz G. and Poortmans J.R., Biochemical Changes in a 100 km Run : Free Amino Acids, Urea and Creatine. European Journal of Applied Physiology, 41 : 61-72, 1979.

Demos M.A., Gitin E.L. and Kagen L.J., Exercise myoglobinuria and acute exertional rhabdomyolysis, Archives of International Medicine, 134 : 669-673, 1974.

deVries H., Prevention of Muscular Distress after Exercise, The Research Quarterly, 32, 2 : 177-185, 1960.

Dickson D.N., Wilkinson R.L. and Noakes T.D., Effects of Ultra Marathon training and Racing on Hematological parameters and Serum Ferritin Levels in Well Trained Athletes, International Journal of Sports Medicine, 3 : 111-117, 1982.

Dohm G.L., Hecker A.L., Brown W.E., Klain G.J., Puente F.R., Askew E.W. and Beecher G.R., Adaption of Protein Metabolism to Endurance Training : Increased Amino Acid Oxidation in Response to Training, Biochemical Journal, 164 : 705-708, 1977.

Dohm G.L., Kasperek G.J. and Barakat H.A., Time Course of Changes in Gluconeogenic Enzyme Activities During Exercise and Recovery, American Journal of Physiology, 249 : E6-E11, 1985.

Dohm G.L., Williams R.T., Kasperek G.J. and Van Pij A.M., Increased Excretion of Urea and N-Methylhistadine by Rats and Humans after a bout of Exercise, Journal of Applied Physiology, 52 : 27-33, 1982.

Dohm G.L., Tapscott E.B. and Kasperek G.J., Protein degradation During Endurance Exercise and Recovery, Medicine and Science in Sports and Exercise, 19, 5 (Suppl.) : S166-S171, 1987.

Dressendorfer R.H. and Wade C.E., The Muscular overuse Syndrome in Long Distance Runners, Physician and Sports Medicine, 11 : 116-130, 1983.

Dufaux B., Assman, G., Order U., Hoederath A and Hollmann W., Plasma Lipoproteins, Hormones, and Energy Substrates During the First Days After Prolonged Exercise, International Journal of Sports Medicine, 2, 4 : 1256-260, 1981.

Eastman Kodak Company, Test Summary for the Kodak DT System, Part no. 14650/C-601, Clinical Products Division, Rochester, New York, 1990.

Einspahr K.J. and Tharp G., Influence of Endurance Training on Plasma Amino Acid Concentrations in humans at Rest and After Intense Exercise, International Journal of Sports Medicine, 10 : 233-236, 1989.

Elia M. and Livesey G., Effects of Ingested Steak and Infused Leucine on Forelimb Metabolism in Man and the Fate of the Carbon Skeletons and Amino Groups of Branched Chain Amino Acids, Clinical Science, 64, : 517-526, 1983.

Farber H.W., Arbetter J., Schaefer E.J., Hill S., Dallal G., Grimaldi R. and Hill N.S., Acute Metabolic Effects of an Endurance Triathlon, Annals of Sports Medicine, 3 : 131-138, 1987.

Farber H.W., Schaefer E.J., Franey R., Grimaldi R. and Hill N.S., The Endurance Triathlon : Metabolic Changes After Each Event and During Recovery, Medicine and Science in Sports and Exercise, 23, 8 : 959-965, 1991.

Felig P., Pozefsky T., Marliss E. and Cahill G.F., Alanine : Key Role in Gluconeogenesis, Science, 167 : 1003-1004, 1970.

Felig P. and Wahren J., Amino Acid Metabolism in Exercising Man, The Journal of Clinical Investigation, 50 : 2703-2714, 1971.

Felig P., Amino Acid Metabolism in Man, Annual Review of Biochemistry, 44 : 933-955, 1975.

Florence J.M., Fox P.T., Planer G.J. and Brooke M.H., Activity, Creatine Kinase and Myoglobin in Duchenne Muscular Dystrophy : A Clue to Etiology?, Neurology, 35, 758-761, 1985.

Fowler W.M., Chowdhury S.R., Pearson C.M., Gardner G., and Bratton R., Changes in Serum Enzyme Levels After Exercise in Trained and Untrained Subjects, Journal of Applied Physiology, 17, 6 : 943-946, 1962.

Fowler W.M., Gardner G.W., Kazerunian H.H. and Lauvstad W.A., The Effect of Exercise on Serum Enzymes, Archives of Physical Medicine and Rehabilitation, 49 : 554-565, 1968.

Fox, E.L. and Mathews, D.K., The Physiological Basis of Physical Education and Athletics, Holt Saunders International Editions, Japan, 1981.

Fox, E.L., Bowers, R.W. and Foss, M.L., The Physiological Basis of Physical Education and Athletics, Wm. C. Brown Publ., Dubuque, Iowa, 1988.

Friden J., Sjostrom M. and Ekblom B., A Morphological Study of Delayed Muscle Soreness, Experientia, 37 : 506-507, 1981.

Friden J., Sjostrom M. and Ekblom B., Myofibrillar Damage Following Intense Eccentric Exercise in Man, International Journal of Sports Medicine, 4 : 170, 1983.

Friden J., Muscle soreness After Exercise : Implications of Morphological Changes, International Journal of Sports Medicine, 5 : 57-66, 1984.

Fulks R.M., Li J.B. and Goldberg A.L., Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm, Journal of Biological Chemistry, 250 : 290-298, 1975.

Galiano F.J., Davis J.M., Bailey S.P., Woods J.A., Hamilton M. and Bartoli W.P., Physiological, Endocrine and Performance Effects of Adding Branched Chain Amino Acids to a 6% Carbohydrate Electrolyte Beverage During Prolonged Cycling, Medicine and Science in Sports and Exercise, 23 (suppl. 4) : S14, 1991.

Garber A.J., Karl I.E. and Kipnis D.M., Alanine and Glutamine synthesis and release from skeletal muscle. Glycolysis and Amino Acid Release, in Journal of Biological Chemistry, 251 : 826-835, 1976.

Gardner G.W., Bratton R., Chowdhury S.R., Fowler W.M. and Pearson C.M., Effect of Exercise on Serum Enzyme Levels in Trained Subjects, Journal of Sports Medicine and Physical Fitness, 4 : 103-110, 1964.

Garlick P.J. and Grant I, Amino Acid Infusion Increases the sensitivity of Muscle Protein Synthesis in vivo to Insulin, Biochemical Journal, 254 : 579-584, 1988.

Gillam I., Skinner S. and Telford R., Effect of antioxidant supplements on indices of muscle damage and regeneration, Medicine and Science in Sports and Exercise, 23 (suppl. 4) : S14, 1991.

Godman G.C., On the Regeneration of Mammalian Striated Muscle, Journal of Morphology, 100 : 27-81, 1957.

Goldberg, A.L. and Chang, T.W., Regulation and Significance of Amino Acid Metabolism in Skeletal Muscle, Federal Proceedings, 37 : 2301-2307, 1978.

Goldberg, A.L. and Odessey R., Oxidation of Amino Acids by Diaphragms, American Journal of Physiology, 223 : 1384-1391, 1972.

Goldstein, L. and Newsholme, E.A., The formation of alanine from amino acids in diaphragm muscle of the rat, Biochemistry Journal, 154 : 555-558, 1976.

Goodman H.M., Stimulatory Action of Insulin on Leucine Uptake and Metabolism in Adipose Tissue, American Journal of Physiology, 206 : 129-132, 1964.

Goodman C., Rogers G.G., Vermaak H. and Goodman M.R., Biochemical Responses During Recovery From Maximal and Submaximal Swimming Exercise, European Journal of Applied Physiology, 54 : 436-441, 1985.

Griffiths P.D., Serum Creatine Kinase and Exercise, British Medical Journal, 2 : 167-168, 1965.

Griffiths P.D., Serum Levels of ATP : Creatine Phosphotransferase (Creatine Kinase). The Normal Range and Effect of Muscular Activity, Clinica Chimica Acta, 13 : 413-420, 1966.

Guyton, A.C., Textbook of medical physiology (6th Edition), W.B. Saunders Co., Philadelphia, 1981.

Hagerman F.C., Kikida R.S., Staron R.S., Sherman W.M. and Costill D.L., Muscle Damage in Marathon Runners, Physical Sportsmedicine, 12 : 39-47, 1984.

Haralambie G., Neuromuscular irritability and Serum Creatine Phosphate Kinase in Athletes in Training, International Zeitschrift Fur Ange Wandte Physiologie Einschliesslich Arbeitsphysiologie, 31 : 279-298, 1973.

Harper, A.E., Benevenga, N.J. and Wohlheuter, R.M., Effects of Ingestion of Disproportionate amounts of Amino Acids, Physiology Reviews, 50 : 248-257, 1970.

Harrison G.G., Buskirk E.R., Carter J.E.L., Johnston F.E., Lohman T.G., Pollock M.L., Roche A.A.F. and Wilmore J., Skinfold measurement and measurement technique. Anthropometric standardization reference manual, Lohman T.G. Roche A.F., Martorell R. (eds), Human Kinetics, Champaign, Illinois, 1988.

Harper A.E., Some Recent Developments in the Study of Amino Acid Metabolism, Proceedings of the Nutrition Society, 42 : 437-439, 1983.

Hederson S.A., Black A.L. and Brooks G.A., Leucine Turnover and Oxidation in Trained Rats During Exercise, American Journal of Physiology 249 : E137-E144, 1985.

Heinemann G., Pabst H. and Schievelbein H, The activation of creatine kinase and the isoenzymes CK-MB in the serum of athletes, Dtsch Z Sportmed, 29 : 33-37, 1978.

Helgheim I., Hetland O., Nilsson S., Ingler F. and Stromme S.B., The Effects of Vitamin E on Serum Enzyme Levels Following Heavy Exercise, European Journal of Applied Physiology, 40, 283-289, 1979.

Highman B. and Altland P.D., Serum Enzyme Rise after Hypoxia and Effect of Anatomic Blockade, American Journal of Physiology, 199 : 981, 1960.

Highman B. and Altland P.D., Effects of Exercise and Training on Serum Enzyme and Tissue Changes in Rats, American Journal of Physiology, 205, 1 : 162-166, 1963.

Hill, A.V., The revolution in muscle physiology, Physiological Reviews, 12 : 56-57, 1932.

Hill A.V., The mechanics of voluntary muscle, Lancet, 24 : 947-951, 1932..

Hodgson P.E., 1989, The Regeneration of Skeletal Muscle, Clinica Chimica Acta, 129, 229-241, 1989.

Holm G., Bjorntorp P. and Jagenburg R., Carbohydrate, Lipid, and Amino Acid Metabolism Following Physical Exercise in Man, Journal of Applied Physiology, 45, 1 : 128-131, 1978.

Holonen P.I. and Konttinen A., Effect of Physical Exercise on Some Enzymes in the Serum, Nature, 193 : 942-945, 1962.

Hough T., Ergographic Studies in Muscular Soreness, American Journal of Physiology, 7 : 76, 1902.

Hunter J.B. and Critz J.B., Effect of Training on Plasma Enzyme Levels in Man, Journal of Applied Physiology, 31, 1 : 20-23, 1971.

Inge K., The Role of Nutrition in Recovery, Fun Runner, Aug-Sept, 25-27, 1990.

Ivy J.L., Muscle Glycogen Synthesis Before and After Exercise, Sports Medicine, 11 (1) : 6-19, 1991.

Iwane H., A Follow-up Study of Changes in Serum Myoglobin and Enzyme Levels in Japanese Triathletes, Annals of Sports Medicine, 3, 2 : 139-143, 1987.

Jones D.A. and Newham D.J., The Effect of Human Muscle Pain and Damage, Proceedings of the Physiological Society, 365 : 76P, 1985.

Jones D.A., Newham D.J., Round J.M. and Tolfree S.E.J., Experimental Human Muscle Damage : Morphological Changes in Relation To Other Indices of Damage, Journal of Physiology, 375 : 435-448, 1986.

Kaman R.L., Goheen B., Patton R. and Raven P., The Effects of Near Maximum Exercise on Serum Enzymes : The Exercise Profile versus The Cardiac Profile, Clinica Chimica Acta, 81 : 145-152, 1977.

Kanter M.M., Kaminsky L.A., La Ham-Saeger J., Lesmes G.R. and Nequin N.D., Serum Enzyme levels and lipid peroxidation in ultra marathon runners, Annals of Sports Medicine, 3 : 39-41, 1986.

Karpati G., Carpenter S. Melmed C. and Eisen A.A., Experimental Ischaemic Myopathic, Journal of Neurological Science, 23 : 129-161, 1974.

Kasperek G.J., Dohm G.L. and Snider R.D., Activation of Branched Chain Keto Acid Dehydrogenase by Exercise, American Journal of Physiology, 248 : R166-R171, 1985.

Kasperek G.J. and Snider R.D., Effect of Exercise Intensity and Starvation on Activation of Branched Chain Keto Acid Dehydrogenase by Exercise, American Journal of Physiology, 252 : E33-E37, 1987.

Keppel. G., Design and Analysis : A researcher's Handbook, 2nd edn. Prentice Hall, Englewood Cliffs, New Jersey, 1982.

Kielblock A.J., Manjoo M., Booyens J. and Katzeff I.E., Creatine Phosphokinase and Lactate Dehydrogenase Levels after Ultra Long Distance Running, South African Medical Journal, 55 : 1061-1062, 1979.

King S.W., Stratland B.E. and Savory J., The Effect of Short Burst of Exercise on Activity Values of Enzymes in Sera of Healthy Young Men, Clinica Chimica Acta, 72 : 211-218, 1976.

Knapik J.J., Wriht J.E., Welch M.J., Patton J.F., Suek L.L., Mello R.P., Rock P.B. and Teves M.A., Changes in Serum Hormones, Substrates, and Enzymes During Prolonged Exercise on Three Consecutive Days, Medicine and Science in Sports and Exercise, 15 : S126, 1983.

Kosano H., Kinoshita T., Nagata N., Takatani O, Isobe M. and Yazaki Y., Change in Concentrations of Myogenic Components of Serum during 93 hours of Strenuous Physical Exercise, Clinical Chemistry, 32, 2 : 346-348, 1986.

Krebs H.A. and Lund P., Aspects of the Regulation of the Metabolism of Branched Chain Amino Acids, Advances in Enzyme Regulation, 15 : 375-393, 1977.

Kreider R., Mitchell M., Miller G., Cortes C. and Miriel V., Effects of Amino Acid Supplementation on Substrate Useage During Ultraendurance Triathlon Performance, Medicine and Science in Sports and Exercise, 23 : 4, S16, 1991.

Kuipers H., Drukker J., Frederik P.M., Guerten P. and Kranenburg G.V., Muscle Degeneration after Exercise in Rats, International Journal of Sports Medicine, 4 : 45-51, 1983.

Kuipers H., Verstappen F., Keizer H., Guerten P. and Kranenburg G., Variability of Aerobic Performance in the Laboratory and its Physiological Correlates, International Journal of Sports Medicine, 6 : 197-210, 1985.

Kuipers H., Keizer H.A., Overtraining in Elite Athletes, Sports Medicine, 6 : 79-92, 1988.

Kuipers H., Jannssen J.M.E., Bosman B., Frederik P.M. and Guerten P., Structural and ultrasonic changes in skeletal muscle associated with long distance training and running (abst.), International Journal of Sports Medicine, 10 (suppl. 3) : S156-S159, 1989.

LaPorta M.A., Linde H.W., Bruce D.L. and Fitzsimons E.J., Elevation of Creatine phosphokinase in Young Men after Recreational Exercise Journal of the American Medical Association, 239, 25 : 2685-2686, 1978.

Le Gros Clark W.E., An Experimental Study of the Regeneration of Mammalian Striped Muscle, Journal of Anatomy, 80 : 24-36, 1946.

Lemon P.W.R. and Nagle F.J., Effects of Exercise on Protein and Amino Acid Metabolism, Medicine and Science in Sports and Exercise, 13 : 141-149, 1981.

Lemon P.W.R., Nagle F.J., Mullin J.P. and Benevenga N.J., In Vivo Leucine Oxidation at Rest and During Two Intensities of Exercise, Journal of Applied Physiology, 53, 4 : 947-954, 1982.

Lemon P.W.R., Yarasheski L.E. and Dolny D.G., The Importance of Protein for Athletes, Sports Medicine, 1 : 474-484, 1984.

Lemon P.W.R., Benevenga N.J., Mullin J.P. and Nagle F.J.,
Effect of Daily Exercise and Food Intake on Leucine Oxidation,
Biochemical Medicine, 33 : 67-76, 1985.

Lemon P.W.R., Chaney M.M., Physiologic Effects of Amino
Acid Supplementation, in Report of the Ross Symposium on
Muscle Development : Nutritional Alternatives to Anabolic
Steroids, Ohio, Ross Labs, 1988.

Lemon P.W.R., Effect of Exercise on Protein Requirements,
Journal of Sports Sciences, 9 (Special Issue): 53-70, 1991a.

Lemon P.W.R., Protein and Amino Acid needs of the
Strength Athlete, International Journal of Sports Nutrition, 1 :
127-145, 1991b.

Lemon P.W.R., Does Exercise alter Dietary Protein
Requirements ?, Advances in Nutrition and Top Sport, Brouns,
Sais and Newsholme (eds) : 15-37, Basel, Karger, 1991c.

Lemon P.W.R. and Proctor D.N., Protein Intake and Athletic
Performance, Sports Medicine, 15 : 313-325, 1991.

Li, J.B. and Jefferson, L.S., Influence of Amino Acid
Availability on Protein Turnover in Perfused Rat Skeletal Muscle,
Biochim Biophys Acta, 544 : 351-359, 1978.

Lijnen P., Hespel P., Fagard R., Lysens R., Vanden Eynde E., Goris M., Goossens W., Lissens W. and Amery A., Indicators of Cell Breakdown in Plasma of Men During and After a Marathon Race, International Journal of Sports Medicine, 9, 2 : 108-113, 1988.

Lindsey D., Navin T. and Finley P., Meaning of Elevated CK-MB, American Heart Journal, 98, 3 : 405-406, 1979.

Magazanik A., Shappiro Y., Meytes D. and Meytes I., Enzyme Blood Levels and Water Balance During a Marathon Race, Journal of Applied Physiology, 36 : 214-217, 1974.

Makatie J. and Teravainen H., Histochemical Studies of Striated Muscle after Temporary Ischemia in the Rat, Acta Neuropath, 37 : 101-109, 1977.

Marieb, E.N., Human Anatomy and Physiology, The Benjamin/ Cummings Publishing Company, Inc., California, U.S.A, 1989.

Maron M.B., Horvath S.M. and Wilkerson J.E., Acute Blood Biochemical Alterations in Response to Marathon Running, European Journal of Applied Physiology, 34 : 173-181, 1975.

Maron M.B., Horvath S.M. and Wilkerson J.E., Blood Biochemical Alterations During Recovery from Competitive Marathon Running, European Journal of Applied Physiology, 36 : 231-238, 1977.

Mason, E.B., Human Physiology, The Benjamin/ Cummings Publishing Company, Inc., California, U.S.A, 1983.

Melamed I., Romen Y., Keren G., Epstein Y. and Dolev E., Myoglobinuria : A Hazard to Renal Function, Archives of International Medicine, 142 : 1277-1279, 1982.

Meltzer H., Mrozak S.Y. and Boyer M., Effect of Intramuscular Injections on Serum Creatine Phosphokinase Activity, Americal Journal of Medical Science, 259 : 42-48, 1970.

Mitchell M., Kreider R., Miller G., Cortes C., and Miriel V., Effects of Amino Acid Supplementation on Metabolic Responses to Ultraendurance Triathlon Performance, Medicine and Science in Sports and Exercise, 23, 4, S15, 1991.

Munjal D.D., McFadden J.A., Matix P.A., Coffman C.D. and Cattaneo S.M., Changes in Serum Myoglobin, Total Creatine Kinase, Lactate Dehydrogenase and Creatine Kinase - MB Levels in Runners, Clinical Biochemistry, 16 : 195-199, 1983.

Musashi Press Release, Amino Acid Handbook, Musashi Pty. Ltd., Victoria, 1991.

National Health and Medical Research Council, Dietary Intakes for use in Australia, Australian Government Printing Service, 1991.

Newham D.H., Mills K.R., Quigley R. and Edwards R.H.T., Muscle Pain and Tenderness after Exercise, Australian Journal of Sports Medicine, 14 : 129-131, 1982.

Newham D.J., Jones D.A., Edwards R.H.T., Large Delayed Creatine Kinase Changes After Stepping Exercise, Muscle and Nerve, 6 : 380-385, 1983.

Noakes T.D. and Carter J.W., Biochemical Parameters in Athletes Before and After Having Run 160 kilometres, South African Medical Journal, 50 : 1562-1566, 1962.

Noakes T.D., Cotzenberg G., McArthur P.S. and Dycman J., Elevated Serum Creatine Kinase MB and Creatine Kinase BB - Isoenzyme fractiona after ultra marathon running , European Journal of Applied Physiology, 52 : 75-79, 1983.

Noakes T.D., Love of Running, Oxford University Press, Capetown, 1986.

Noakes T.D., Effect of Exercise on Serum Enzyme Activity in Humans, Sports Medicine, 4 : 245-267, 1987.

Norregaard Hansen K., Bjerre-Knudsen J., Brodthagen U., Kordal R. and Paulev P.E., Muscle Cell Leakage due to Long Distance Training, European Journal of Applied Physiology, 48 : 177-188, 1982.

Nuttall F.Q. and Jones B., Creatine Kinase and Glutamic Oxalacetic Transaminase activity in serum : Kinetics of Change with exercise and effect of Physical Conditioning, Journal of Laboratory and Clinical Medicine, 71, 5 : 847-854, 1968.

Odessey R., Khairallah E.A., Goldberg A.L., Origin and Possible Significance of Alanine Production by Skeletal Muscle, The Journal of Biological Chemistry, 249, 23 : 7623-7629, 1974.

Olerud J.E., Homer L.D., Carroll H.W., Incidence of Acute Exertional Rhabdomyolysis : Serum Myoglobin and Enzyme levels as indicators of Muscle Injury, Archives of Internal Medicine, 136 : 692-697, 1976.

Ono I., Studies on Myoglobinuria. Tohoku, Journal of Experimental Medicine, 57 : 273-281, 1953.

Palevsky H.I., Douglas P.S., Hiller W.D., Bogin K., Reichek N. and O'Toole M., Muscle Enzyme Patterns Before and After Ultra Endurance Racing, Medicine and Science in Sports and Exercise, 18 : S60, 1986.

Paul A.A., Southgate A.A.T. and Russell J., The First Supplement to McCance and Widdowson's - The Composition of Foods, Her Majesty's Stationary Office, London, 1988.

Paxton R. and Harris R.A., Regulation of Branched Chain Alpha-Ketoacid Dehydrogenase Kinase, Archives of Biochemistry and Biophysics, 231, 1 : 48-57, 1984.

Pearce J., Serum Creatine Kinase and Exercise, British Medical Journal, 2 : 167, 1965.

Poortmans J.R., Siest G., Galteau M.M. and Houot O., Distribution of Plasma Amino Acids in Humans during Submaximal Prolonged Exercise, European Journal of Applied Physiology, 32 : 143-147, 1974.

Poortmans J.R. and Haralambie G., Biochemical Changes in a 100km Run : Proteins in Serum and Urine, European Journal of Applied Physiology, 40 : 245-254, 1979.

Poortmans J.R., Use and Usefulness of Amino Acids and Related Substances during Physical Exercise, Biochemical Aspects of Physical Exercise, Benzi G., Packer L. and Siliprandi N. (eds.), New York, Elsevier : 285-294, 1986.

Poortmans J.R., The Role of Global Protein Metabolism and the Catabolism of Amino Acids during Muscular Exercise, Hermes, 21, (2-3) : 369-386, 1990.

Raimondi G.A., Puy R.J.M., Raimondi A.C., Schwarz E.R. and Rosenberg M., Effects of Physical Training on Enzymatic Activity of Human Skeletal Muscle, Biomedicine, 22 : 496-501, 1975.

Rasch P.J. and Schwartz P.L., Effect of Amateur Wrestling on Selected Serum Enzymes, Journal of Sports Medicine and Physical Fitness, 12 : 82-86, 1972.

Refsum H.E., Gjessing L.R. and Stromme S.B., Changes in Plasma Amino Acid Distribution and Urine Amino Acid Distribution and Urine Amino Acids Excretion During Prolonged Heavy Exercise, Scandinavian Journal of Clinical and Laboratory Investigation, 39, : 407-413, 1979.

Reinhart W.H., Staubli M., Kochli H.P. and Straub P.W., Creatine Kinase and MB-Fraction after a long distance race, Clinica Chimica Acta, 125 : 307-310, 1982.

Reinhart W.H., Staubli M. and Straub P.W., Impaired Red Cell Filter Ability with Elimination of old Red Blood Cells during a 100 km. race., Journal of Applied Physiology, 54 : 827-830, 1983.

Rennie M.J., Edwards R.H.T., Davies C.T.M., Krywawych S., Halliday D., Waterlow J.C. and Millward D.J., Protein and Amino Acid Turnover During and After Exercise, Biochemical Society Transactions, 8 : 499-501, 1980.

Rennie M.J., Edwards R.H.T., Krywawych S., Davies C.T.M., Holliday D., Waterlow J.C. and Millward D.J., Effect of exercise on protein turnover in man, Clinical Science, 61 : 627-634, 1981.

Richterich R., Rosin S. and Aepli U., Progressive Muscular Dystrophy : The Identification of the Carrier State in the Duchenne Type by Serum Creatine Kinase Determination, American Journal of Human Genetics, 15 : 133-154, 1963.

Riley W. J., Pyke F.S., Roberts A.D. and England J.F., The Effect of Long Distance Running on Some Biochemical Variables, Clinica Chimica Acta, 65 : 83-89, 1975.

Robinson D., Williams P.T., Worthington D.J. and Carter T.J.N., Raised Creatine Kinase Activity and Presence of Creatine Kinase MB Isoenzyme After Exercise, British Medical Journal, 285 : 1619-1620, 1982.

Rogers M.A., Apple F.S., and Stull G.A., Creatine Kinase Isoenzyme Elevations and Clearance Rates in Men and Women Following a 42.2 Km. Race, Medicine and Science in Sports and Exercise, 23, 4 : S15, 1991.

Roti S., Iori E., Guiducci U., Emanuele R., Robuschi G., Bandini P., Gnudi A. and Roti E., Serum Concentrations of Myoglobin, Creatine Phosphokinase and Lactic Dehydrogenase after Exercise in Trained and Untrained Athletes, Journal of Sports Medicine, 21 : 113-118, 1981.

Sabria M., Ruibal A., Rey C., Foz M. and Domenech F.M., Influence of Exercise on Serum levels of Myoglobin Measured by Radioimmunoassay, European Journal of Nuclear Medicine, 8 : 159-161, 1983.

Saks V.A., Rosenshtaukh L.V., Smirnov V.N. and Chazov E.I., Role of creatine phosphokinase in cellular function and metabolism, Canadian Journal of Physiology and Pharmacology, 56 : 691-706, 1978.

Saltin B. and Gollnick P.D., Skeletal Muscle Adaptability : Significance for Metabolism and Performance, Hanbbook of Physiology, 10, 19 : 555-631, 1983.

Sanders T.M. and Bloor C.M., Effects of Repeated Endurance Exercise on Serum Enzyme Activities in Well Conditioned Males, Medicine and Science in Sports and Exercise, 7 : 1 : 44-47, 1975.

Schnohr P., Grande P. and Christiansen C., Enzyme Activities in Serum after Extensive Exercise, with Special Reference to Creatine Kinase MB, Acta Med. Scand., 208 : 229-231, 1980.

Schwane J.A., Johnson S.R., Vandenakker C.B. and Armstrong R.B., Delayed- onset muscular soreness and Plasma CPK and LDH activities after Downhill Running, Medicine and Science in Sports and Exercise, 15, 1 : 51-56, 1983.

Schwane J.A. and Armstrong R.B., Effect of Training on Skeletal Muscle Injury from Downhill Running in Rats, Journal of Applied Physiology, 55 : 969-975, 1983.

Sharp R., Vukovich M., King D., Kesi L. and Schaulis D.,
Effects of a Commercial Amino Acid Supplement on Adaptions to
Cycle Ergometer Training, Medicine and Science in Sports and
Exercise, 23, (suppl. 4) : S15, 1991.

Sherman W.M., Costill D.L., Fink W.J. and Miller J.M.,
Effect of Exercise-Diet Manipulation on Muscle Glycogen and its
Subsequent Utilization during performance, International Journal
of Sports Medicine, 2 :114-118, 1981.

Showell C., Personal Communication, Head of the
Pathology Department, Launceston General Hospital, November,
1992.

Shumate J.B., Brooke M.H., Carroll M.D. and Davis J.E.,
Increased Serum Creatine Kinase after Exercise : A Sex Linked
Phenomenon, Neurology, 29 : 902-904, 1979.

Stiegel A.J., Silverman L.M. and Holman L., Elevated
Creatine Kinase MB Isoenzyme Levels in Marathon Runners,
Journal of the American Medical Association, 246, 18 : 2049-
2051, 1981.

Siegel A.J., Silverman L.M. and Evans W.J., Elevated Skeletal Muscle Creatine Kinase MB Isoenzyme Levels in Marathon Runners, Journal of the American Medical Association, 250 : 20, 2835-2837, 1983.

Simonsen, E. and Lind, A., Physiology of work capacity and fatigue, Charles C Thomas, Springfield, U.S.A, 1971.

Sjodin B., Thorstensson A., Frith K. and Karlsson J., Effect of Physical Training on LDH activity and LDH isoenzyme Pattern in Human Skeletal Muscle, Acta Physiol. Scand., 97 : 150-157, 1976.

Sjodin B., Lactate Dehydrogenase in Human Skeletal Muscle, Acta Physiol. Scand., S436 : 1- 27, 1976.

Slavin J.L., Lanners G. and Engstrom M.A., Amino Acid Supplements : Beneficial or Risky ?, Sports Coach, Oct-Dec : 43-45, 1983.

Smith R.F., Exertional Rhabdomyolosis in Naval Officer Candidates, Archives of International Medicine, 121 : 313-319, 1968.

Snell K. and Duff D.A., The release of Alanine by Rat Diaphragm Muscle in Vitro, Biochemical Journal, 162 : 399-403, 1977.

Stansbie D., Aston P., Dallimore N.S., Williams H.M.S. and Willis N., Effect of Exercise on Plasma Pyruvate Kinase and Creatine Kinase Activity, Clinica Chimica Acta, 132 : 127-132, 1983.

Steele B.W., Gobel F.L., Nelson R.R., Yasmineh W.G., Creatine Kinase Isoenzyme Activity following Cardiac Catheterisation and Exercise Stress Testing, Chest, 73, 4 : 489-496, 1978.

Stephens, J.A. and Taylor, A., Fatigue of maintained voluntary muscle contraction in man, Journal of Physiology, 220 : 1-18, 1972.

Stone M.H., Keuth R.E., Kearney J.T., Fleck S.J., Wilson G.D. and Triplett N.T., Overtraining : A Review of the Signs, Symptoms and Possible Causes, Journal of Applied Sports Science Research, 5, 1 : 35-50, 1991.

Strachan A.F., Noakes T.D., Kotzenberg G., Nel A.E. and De Beer F.C., C-Reactive Protein Concentrations during Long Distance Running, British Medical Journal, 289 : 1249-1251, 1984.

Telford R.D., Regeneration - A Nutritional Perspective, Excel, 6, 2 : 2-5, 1990.

Thomson W.H.S., Sweetin J.C. and Hamilton I.J.D., ATP and Muscle Enzyme Efflux After Physical Exertion, Clinica Chimica Acta, 59 : 241-245, 1975.

Tludus P.M. and Ianuzzo C.D., Effects of Intensity and Duration of Muscular Exercise on Delayed Soreness and Serum Enzyme Activities, Medicine and Science in Sports and Exercise, 15, 6 : 461-465, 1983.

Triplett N.T., Stone M.H., Adams C., Allran K.D. and Smith T.W., Effects of Asparic Acid Salts on Fatigue Parameters during Weight Training Exercise and Recovery, Journal of Applied Sport Science Research, 4, 4 : 144-147, 1990.

Vandewalle L., Wagenmakers A.J.M., Smets K., Brouns F. and Saris W.H.M., Effect of branched chain amino acid supplements on exercise performance in glycogen depleted subjects (abst.), Medicine and Science in Sports and Exercise, 23, (suppl.4) : S116, 1991.

Vejjajiva A. and Teasdale D.M., Serum Creatine Kinase and Physical Exercise, British Medical Journal, 1 : 1653, 1965.

Viru A., Mobilisation of Structural Proteins during Exercise, Sports Medicine, 4 : 95-128, 1987.

Wagenmakers A.J.M., Salden H.J.M. and Veerkamp J.H., The Metabolic Fate of Branched Chain Amino Acids and 2-Oxo Acids in Rat Muscle Homogenates and Diaphragms, International Journal of Biochemistry, 17, 9 : 957-965, 1985.

Wagenmakers A.J.M., Coakley J.H. and Edwards R.H.T., Exercise Induced Activation of the Muscle Branched Chain 2-Oxo Acid Dehydrogenase in Patients with McCardles Disease, Clinical Science, 73, (Suppl. 17) : 1P, 1987.

Wagenmakers A.J.M., Brookes J.H., Coakley J.H., Reilly T. and Edwards R.H.T., Exercise Induced Activation of the Branched Chain 2-Oxo Acid Dehydrogenase in Human Muscle, European Journal of Applied Physiology, 59 : 159-167, 1989.

Wagenmakers A.J.M., Coakley J.H. and Edwards R.H.T., Metabolism of Branched Chain Amino Acids and ammonia During Exercise : Clues from McArdles Disease, International Journal of Sports Medicine, 11, S2 : S101-S113, 1990.

Wahren J., Felig P., Hendler R. and Ahlborg G., Glucose and Amino Acid Metabolism During Exercise After Recovery, Journal of Applied Physiology, 34, 6 : 838-845, 1973.

Wells C.L., Stern J.R. and Hecht L.H., Hematological Changes following a Marathon Race in Male and Female Runners, European Journal of Applied Physiology, 48 : 41-49, 1982.

White T.P. and Brooks G.A., Glucose, Alanine and Leucine Oxidation in Rats at Rest and Two Intensities of Running, American Journal of Physiology, 240 : E155-E165, 1981.

Wolfe R.R., Godenough R.D., Wolfe M.H., Royle G.T. and Nadel E.R., Isotopic analysis of leucine and urea metabolism in exercising humans, Journal of Applied Physiology, 52 : 458-466, 1982.

Woodard J.G. and Town G.P., The progressive Metabolic and Energy Demands of Triathlon Competitors, Medicine and Science in Sports and Exercise, 15 : S126, 1983.